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Investigating DNA Barcoding Potentials and Genetic Structure in Ozobranchus spp. from Atlantic and Pacific Ocean Sea Turtles

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INVESTIGATING DNA BARCODING POTENTIALS AND GENETIC STRUCTURE IN *OZOBRANCHUS* SPP. FROM ATLANTIC AND PACIFIC OCEAN SEA TURTLES

A thesis submitted in partial fulfillment of the
requirements for the degree of
Master of Science

By

TRIET MINH TRUONG
B.S., Wright State University, 2011

2014
Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

February 6, 2014

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Triet Minh Truong ENTITLED Investigating DNA barcoding potentials and genetic structure in *Ozobranchus* spp. from Atlantic and Pacific Ocean sea turtles BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

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ABSTRACT

Truong, Triet Minh. M.S. Department of Chemistry, Wright State University, 2014. DNA Barcoding: A Novel Tool for Investigating Genetic Structure in *Ozobranchus* spp. from Atlantic and Pacific Ocean Sea Turtles.

The Ozobranchidae family is the smallest and least studied hirudinean taxon. Our research includes the largest molecular dataset yet reported for marine ozobranchids (*Ozobranchus margoi* and *Ozobranchus branchiatus*) with the most number of documented turtle hosts (57) from the Atlantic and Pacific Oceans to date of any marine turtle epibiont study. Turtle species sampled in this study include loggerheads (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), olive ridley (*Lepidochelys olivacea*), and green turtles (*Chelonia mydas*). Phylogenetic analyses of mitochondrial (COI) and nuclear ribosomal (18S and 28S) genes all support the monophyly of marine Ozobranchidae leeches with speciation occurring over an extensive period of time, likely prior to the Isthmus of Panama. Histone H3 data suggests at least three histone H3 genes for *O. margoi*. In addition, mtDNA analyses show higher genetic structure in the Atlantic for *O. branchiatus* existing in both ocean basins. The small tropical family of turtle annelids was also used to examine the limitations of DNA barcoding on taxa with incomplete taxonomic sampling and to assess whether these issues can be adequately resolved using the character-based approach. The ability to assign ocean basin origin of leech specimens using character-based DNA barcoding suggests the potential for this tool to be integrated with other applications besides species identification.

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I. INTRODUCTION

The rising emergence of sea turtle diseases has increased awareness of the importance of better understanding how epibionts affect the health and ecology of their turtle hosts (Bunkley-William *et al*. 2008; Lazo-Wasem *et al*. 2007; Lazo-Wasem *et al*. 2011). Sea turtle leeches (*Ozobranchus* spp.) have received attention recently due to their association with the neoplastic disease fibropapillomatosis (FP) (Greenblatt *et al*. 2004). Although heavy leech infestation is not necessarily an indicator of the host specimen's health status, there have been reported cases over the years of detrimental and even fatal injuries on captive marine turtles caused by the superinfection of these leeches (Davies & Chapman 1974; Schwartz 1974). A recent study referred to these documented infections as sea turtle leech erosion disease (SLED) and reported the first wild case of SLED on a hawksbill turtle (*Eretomochelys imbricata*) (Bunkley-Williams *et al*. 2008).

Only two leeches (*Ozobranchus branchiatus* and *Ozobranchus margoi*) are known to be associated with sea turtles (Sawyer *et al*. 1975), and limited attention has been paid to identifying the leech species, a potential vector in FP (Williams *et al*. 2006). Furthermore, in documented cases of leech superinfection, only a subsample of the *Ozobranchus* spp. population is identified (Bunkley-Williams *et al*. 2008). Additionally, identifying species of leech can be difficult due to the leeches' small size (2 mm to 23 mm in length) and varied larval and cocoon life stages. Most studies have employed adult specimens. Yet, parasite etiology requires accurate species identification at all life stages. Marine Ozobranchidae leeches are believed to complete their entire lifecycle on their turtle host although this still awaits validation (Williams *et al*. 1994; Sawyer *et al*. 1975). If host specificity is indeed restricted to a single individual host throughout the entire leech lifecycle, then any evidence of population structure across both ocean basins is an indication of the potential for host-parasite co-evolution.

Marine Ozobranchidae leeches have been documented on all species of sea turtles with the exception of flatback (*Natator depressus*) and leatherback (*Dermochelys coriacea*) turtles (Bunkley-Williams *et al*. 2008; McGowin *et al*. 2011). Recently, *Ozobranchus* spp. have been documented for the first time in Iran (*C. mydas*), Taiwan (*C. caretta*, *C. mydas*, and *E. imbricata*) and Brazil (*C. caretta*)(Kami *et al*. 2007; Rodenbusch *et al*. 2012; Tseng & Cheng 2013). This study reports the first findings of *O. margoi* on Brazilian *C. mydas* turtles and the second to document multiple *Ozobranchus* spp. species on a *C. mydas* (McGowin *et al.* 2011). The cosmopolitan distribution of Ozobranchidae leeches make them ideal candidates for studying host-parasite relationships and their effects on turtle health and ecology.

Genetic techniques for species identification have become the most utilized molecular approach in parasitology due to the limited morphological attributes and indistinguishable life stages of parasites (Criscione *et al*. 2005; McManus & Bowles 1996). A widely published DNA-based method of species identification is DNA barcoding. The basis behind DNA barcoding involves targeting selected segments of DNA (standardized molecular markers) that are known to have relatively few insertions or deletions. In addition, the DNA region must also have high interspecific variation but minimal intraspecific genetic differences (Waugh 2007). As a result, mitochondrial DNA (mtDNA) is often a preferred choice for barcoding purposes over nuclear genes (Waugh 2007).

Mitochondrial cytochrome *c* oxidase I (COI) gene is perhaps the most popular DNA barcoding genetic marker with numerous studies supporting it as the ideal standardized DNA region for establishing a global taxon identification system (Hebert *et al*. 2003;

Ratnasingham & Hebert 2007). Simple character-based DNA barcoding using COI has been successfully employed to identify both *O. branchiatus* and *O. margoi* at all stages of development from Florida loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles (McGowin *et al*. 2011). In order to confirm the effectiveness of the COI barcode to distinguish marine leeches and to test whether the locus can accurately assign ocean basin origin, additional samples were analyzed from different sampling locations of the Atlantic and the Pacific Oceans. This comprehensive barcoding effort makes possible the unambiguous identification of *Ozobranchus* spp. at all stages of their lifecycle, which is essential for ectoparasite studies.

Our focus is not simply to identify marine leeches using DNA barcoding but integrate DNA barcoding with those of evolutionary biology and population genetics. Until now, the phylogeny of *Ozobranchus* spp. from a genetic perspective has been studied primarily on mitochondrial data with limited studies using nuclear ribosomal 18S as a secondary molecular marker (Apakupakul *et al*. 1999; Light & Siddall 1999; Siddall & Burreson 1998). Prior to the recent publications on *O. branchiatus* (Lavretsky *et al*. 2012; McGowin *et al*. 2011), *O. margoi* was the only Ozobranchidae leech with molecular data available in the National Center for Biotechnology Information (NCBI) GenBank. Thus, past molecular studies on leech phylogeny underrepresent marine turtle leeches (Utevsky & Trontelj 2004; Utevsky *et al*. 2007; Williams & Burreson 2006).

This study presents the largest molecular data set assembled for genetic studies of *Ozobranchus* spp. with the most documented number of turtle host species. The diverse global distribution of samples enables a broad assessment of the species' genetic variation using mtDNA (COI), nuclear ribosomal (18S rDNA and the D1 region of 28S rDNA), and nuclear protein coding-genes (histone H3). Slowly evolving genes, like rRNA, are essential genetic markers needed for recovering ancient relationships, providing insights beyond species identification (Woese 2000). The information obtained in this study offers the first comprehensive report on the evolutionary relationships of marine turtle leeches and will help evaluate the potential of DNA barcoding as a novel tool for determining parasite geographic origin.

II. MATERIAL AND METHODS

Sampling, identification, and DNA extraction

O. branchiatus and *O. margoi* at all stages of development were collected from marine turtles in the Pacific and Atlantic Oceans. Species of sea turtle sampled included *E. imbricata* (Barbados), *C. mydas* (Florida, Hawaii, Hong Kong, and Brazil), *C. caretta* (Florida), and olive ridley (*Lepidochelys olivacea*) from eastern Pacific Mexico. *O. branchiatus* was found in both ocean basins, while *O. margoi* was only sampled in the Atlantic Ocean. It is important to note, a *C. mydas* identified off the coast of southern Brazil was found with both leech species. This is the second study to document an infection of a sea turtle with both *O. branchiatus* and *O. margoi* (McGowin *et al*. 2011). Cocoon or larval samples analyzed for species identification purposes were collected from Hawaiian sea turtles and a Florida *C. caretta*. Although a majority of the *Ozobranchus* spp. specimens were collected from live captured marine turtles, some samples were obtained from dead Hawaiian sea turtles washed ashore on the beach. Leeches collected from *L. olivacea* were provided by Eric A. Lazo-Wasem (Peabody Museum of Natural History, New Haven, CT). Methodology for sampling leeches, morphological identification, and DNA extraction of *Ozobranchus* spp. specimens are given in McGowin *et al*. (2011). Appendix S1 lists the source of all *Ozobranchus* spp. samples analyzed along with their associated turtle hosts and GenBank accession numbers for COI and nuclear ribosomal gene sequences.

PCR amplification and sequencing

All samples reported in Appendix S1 have been sequenced for COI. Sequencing at non-mtDNA loci was conducted only on samples that have previously been sequenced for COI either in this study or McGowin *et al.* (2011). PCR amplification and sequencing of COI and histone H3 followed McGowin *et al.* (2011) and Lavretsky *et al.* (2012), respectively. PCR and sequencing work for 18S and 28S differ from histone H3 only in that the specific annealing temperature was 52°C and the temperature setting time was one minute at 72°C. Two direction sequencing was done on all samples, except for two unpooled *O. margoi* specimens noted in Appendix S1. The PCR primers employed in this study are listed in Table 1.

Genetic data of previous *Ozobranchus* spp. studies (McGowin *et al.* 2011) all originated from pooled samples of the same species based on preliminary morphological assessment of adults on the same host. Several Florida and Hawaii specimens in this study were also pooled in order to obtain greater tissue mass for DNA extraction. This was later determined to be unnecessary, so in order to evaluate the probability that pooling might overestimate genetic variability, additional sequences at the two largest molecular markers examined in this study (COI and 18S) were obtained from unpooled samples using specimens collected at the same location and from the same turtle host or at least identical turtle host species as the leeches used in the pooled samples. However, due to low sampling size, genetic data from *O. branchiatus* specimens collected on a Florida loggerhead turtle could only be obtained from a single pooled sample of the only two available leeches. Since comparative analysis of pooled and unpooled samples revealed no mtDNA genetic difference with minimal variability present at 18S, all sequencing data from pooled samples were included in this study.

Alignment analyses and genetic diversity

Alignment analysis of genetic sequences was done using SequencherTM 4.9 (Gene Codes, Inc.) and MEGA5 (Tamura *et al*. 2011). All sequences were aligned using Muscle v3.7 (Edgar 2004) under default options. Correlation coefficients (Mantel tests) were computed using the Alleles in Space (AIS) software (Miller 2005) to determine the significance between genetic and geographical distances. GPS coordinates that were not acquired in the field were obtained from Google Maps in order to compute geographical distances in AIS. Additional simple and partial Mantel tests were performed in zt version 1.1 (Bonnet & Van de Peer 2002) to assess the possibility of host-parasite coevolution. Median-joining networks were generated using Network 4.6.1.1 (Bandelt *et al*. 1999) and DNA sequence diversity, including F-statistics, were calculated using DnaSP v5 (Librado & Rozas 2009). Fu's F*s* (Fu 1997) and Tajima's D (Tajima 1989) values were computed in DnaSP v5 based upon the total number of mutation and total number of segregating sites, respectively. The number of variable nucleotide and amino acid sites in the data set along with distance analyses were conducted in MEGA5. Pairwise sequence divergences analyzed at species and genus levels and standard errors were calculated using a Kimura 2-parameter (K2P) distance model (Kimura 1980) rather than a more realistic model for comparisons with canonical and taxa related distance-based barcoding studies (Hebert *et al*. 2003; Hebert *et al*. 2004; Reid *et al*. 2011). The rate variation among sites was modeled with a gamma distribution (shape parameter = 5) and all positions containing gaps and missing data were eliminated.

Phylogenetics and character-based barcoding

Molecular phylogenetic trees generated in MEGA5 with node support were evaluated using 1000 bootstrap replicates. Neighbour-joining (NJ) trees were conducted under the same K2P model settings as those used for distanced-based barcoding analyses, while phylogenetic relationships were explored using Maximum Likelihood (ML) bootstrap consensus trees based on the most realistic substitution models, such as General Time Reversible for COI (Nei & Kumar 2000) and K2P for 18S and 28S. Gaps were treated with the Pairwise-Deletion option for unambiguous alignments or alignments with minimal gaps and missing data (COI and histone H3). Alignments with significant areas of uncertainties (28S and 18S rDNA) were treated with the Complete-Deletion option. Appendix S4 to Appendix S6 provide the GenBank accession numbers of all non-*Ozobranchidae* taxa used for phylogenetic purposes. Character-based DNA barcoding methods followed McGowin *et al*. (2011) with the exception that the first position in the barcode is designated to be the first alignment position free of gaps. Character-based analysis was performed manually by analyzing polymorphic sites within MEGA5 because the selected taxa groups contained minimal polymorphic sites and sequences, which eliminated the need for software.

III. RESULTS

Genetic diversity of mtDNA barcoding gene

COI served as the barcoding locus for marine turtle leeches. A total of 72 COI sequences, including GenBank sequences, were obtained (16 pooled; 56 unpooled) from 109 individuals (658 bp; 219 amino acids) with 126 variable sites (19% of the nucleotide positions). Nucleotide composition showed a bias against C and G. Only 2% of amino acid positions were variable (Table 2), which is consistent with previous findings on Ozobranchidae leeches (McGowin *et al*. 2011). The *O*. *margoi* sequence by Siddall & Burreson (1998) (GenBank [accession number AF003268]) was identical to those in this study and McGowin *et al*. (2011) but it was not included in any analyses because of a missing single nucleotide position, suggesting possible sequencing errors.

Population structure and network

All COI haplotypes were incorporated into a single median-joining network distinguished by species and ocean basin (Fig. 1). No haplotypes were shared between the two species, and none were shared among *O. branchiatus* on different species of sea turtles (*C. caretta*, *C. mydas*, and *L. olivacea*) or on turtles from different sampling sites (Table 3). Prior to this study, all available *O. branchiatus* GenBank sequences matched haplotype OB-A1 with the exception of haplotype OB-A2 (McGowin *et al*. 2011). No new haplotypes were identified for *O. margoi* assuming that sequencing errors contributed to genetic differences in data from past studies.

Ten haplotypes were identified for *O. branchiatus* (four in the Atlantic and six in the Pacific) (Table 3), but COI show no variation in all *O. margoi* samples regardless of host (*C. caretta*, *C. mydas*, and *E. imbricata*) or location (Florida, Barbados, and Brazil) (Fig. 1). COI analysis of *O. branchiatus* specimens reveal significant population structure with F_{ST} estimates ranging from 0.8548 to 0.9774 in the Pacific, 0.9363 in the Atlantic, and 0.6995 between ocean basins. These values are comparable to the F_{ST} estimate computed between the two separate species of leech (0.9438), suggesting possible population isolation for *O. branchiatus*. Altogether 45 polymorphic sites (substitutions) and a total of 47 mutations accounted for the genetic variation within *O. branchiatus*. Table 3 displays the K2P pairwise divergences along with the total number of mutated positions between the different *O. branchiatus* haplotypes. Appendix S2 supplements Table 3 by giving a complete listing of each individual mutated position separating those haplotypes from one another. The number of mutations differentiating the haplotypes within this species range from one to 28.

Median-joining network analyses revealed deep division between the two sister species with the Atlantic *O. margoi* (OM-A1) and the northern Atlantic *O. branchiatus* haplotypes (OB-A3) connected through 100 mutated positions (substitutions). One specimen has been found with haplotype OB-A3. All other *O*. *branchiatus* samples collected from *C*. *mydas* foraging in the northern Atlantic region shared the dominant haplotype OB-A1, separated from OB-A3 by a single mutation. The rarity of OB-A3 suggests this random mutation has not yet been fixed within *O*. *branchiatus* populations above the Atlantic equatorial region. Similarly, in the central Pacific realm, the dominant *O*. *branchiatus* haplotype (OB-P1) parasitizing *C*. *mydas* of the Hawaiian archipelago also differed from the two less prevalent haplotypes (OB-P2 and OB-P3) by one mutation, suggesting perhaps most haplotypes originated recently and is indicative of a population expansion (Ferreri *et al*. 2011).

Although the *O*. *branchiatus* haplotype diversity was lower in the Atlantic, genetic divergence was more profound and extensive with nucleotide diversity being nearly four-fold higher compared to the Pacific populations (Table 4). Sampling size was concentrated predominately at two locations: Hawaii and Florida. Analysis of Florida samples revealed significant negative values for Tajima's D (-2.52366) but not for Fu's F*s* (4.139), while Hawaiian samples resulted in significant deviations for Fu's F*s* (-0.775) but not for Tajima's D (-0.80383). Intraspecific analysis of COI revealed a sharp separation (19 substitutions) between the two distinct haplotypes (OB-A1 along the coast of Florida and OB-A4 off the coast of southern Brazil) characterizing the northern and southern Atlantic *C*. *mydas* leeches. In contrast, leeches from geographically distinct Pacific *C*. *mydas* populations were characterized by more shallow divisions with the Hawaiian and western Pacific (Hong Kong) specimens separated by a maximum of nine mutations.

Besides geography, host specificity appears to also play a role in shaping population structure for the *O*. *branchiatus* leech. Once again, this factor emerged more prominently in the Atlantic Ocean. Northern Atlantic *C*. *caretta* and eastern Pacific Mexican *L*. *olivacea* turtles were the only other turtle species besides *C*. *mydas* with *O*. *branchiatus* sampled in this study. Due to limited sampling size, it is not certain which of the two haplotypes (OB-P4 and OB-P6) from the *L. olivacea* leeches were the dominant haplotype in the eastern Pacific population. However, both differed by only a single substitution with the OB-P4 haplotype having greater genetic distance from the Hawaiian and Western Pacific haplotypes by one mutation.

Originally documented in McGowin *et al*. (2011), the two pooled leeches from a *C. caretta* raised the question of whether cryptic specimens could exist in the Atlantic basin. This possibility is further suggested by the *C. caretta* leeches' deep level of divergence from all other *O. branchiatus* COI haplotypes in this study (Fig. 1, Table 3). Interestingly, mtDNA of the *C. caretta* leeches and the southern Atlantic specimens exhibit a greater mutation break from the northern Atlantic *O. branchiatus* population than the other populations in the Pacific. Hence, even though genetic structure is present for *O. branchiatus* in both the Atlantic and Pacific, the unexpected higher intraspecific variation in the Atlantic implicate greater structure exists in this ocean basin.

Genetic diversity of nuclear ribosomal loci

Due to minimal intraspecific variation, sequencing of nuclear ribosomal 28S was limited to Florida (*C. caretta* and *C. mydas*), Barbados (*E. imbricata*), and Hawaiian (*C. mydas*) specimens. No sharing of haplotypes occurred between the two species with five nucleotide differences (~1.5 to 1.8 divergence) separating *O. branchiatus* and *O. margoi* at positions 58, 83, 171, 176, and 207. Nucleotide differences at positions 147 and 204 are due to heterozygosity in the *O. margoi* specimens. Although all 28S sequences for *O. branchiatus* came from pooled samples, no heterozygosity was detected. On the other hand, heterozygosity was present in all *O. margoi* 28S sequences, except for a single pooled sample from a Florida *C. mydas*.

Nuclear ribosomal gene 18S was characterized by higher intraspecific variation with an average 1.11 % interspecific divergence. All samples were homozygous for *O. branchiatus* with three haplotypes identified. Genetic variation (average 0.05 %) within *O. branchiatus* was limited to only two polymorphic positions and haplotype OB-18S1 was the only haplotype restricted to one ocean basin (Atlantic) and one species of marine turtle (*C. mydas*) (Fig. 1). Leeches from western Pacific *C. mydas* (Hong Kong) and northern Atlantic *C. caretta* shared haplotype OB-18S2, while those collected from Mexican *L. olivacea* and from *C. mydas* in Brazil and Hawaii shared OB-18S3 (Fig.1).

O. margoi samples were heterozygous at the 18S locus with a total of five heterozygous positions in the 18S alignment. The five heterozygous positions all consisted of the same ambiguity (Y). Position 1621 was the only site to have a fixed ambiguity in all the samples indicating perhaps the presence of a duplicate 18S gene or amplification of a secondary artifact during the PCR process. Although intraspecific variations were lost upon removal of heterozygous positions, interspecific differences were still present to show speciation between the two species. A BLAST search resulted in a 99 % match, but Apakupakul *et al*. (1999) 18S sequence (GenBank [accession number AF003268]) was not used for any genetic analyses due to several alignment gaps with the 18S sequences in this study.

The two reverse PCR primers described in Lavretsky *et al*. (2012) were developed to be compatible with the Cogan *et al*. (1998) forward primer. Those primers successfully amplified the histone H3 genes (GenBank accession numbers KF728228 and KF728229) for the pooled *C. caretta*-*O. branchiatus* sample originally documented in McGowin *et al*. (2011). Comparative phylogenetic analysis to the other histone H3 sequences from Lavretsky *et al*. (2012) revealed the *C. caretta* leeches to be sister taxa to other *O. branchiatus* specimens from Florida at both histone H3 loci (H3R1 and H3R2). The same histone H3

PCR primers were used for *O. margoi*, but sequencing results indicated more specific primers were needed to separate the nuclear protein-coding genes. As a result, two forward primers compatible with the Cogan *et al*. (1998) reverse primer were developed in this study to amplify histone H3 in *O. margoi*: OM-H3R1 (5′-GTGAAAAGGCTCCTAGG AAA-3′) and OM-H3R2 (5′-GTGGAAAGGCACCTAGGAAG-3’). Sequencing results, however, still yielded multiple products, suggesting the possibility of at least three histone H3 genes rather than two for this second species of marine leech.

Geographic and genetic distances correlation

The effectiveness of distance-based DNA barcoding relies heavily on accurate assessment of genetic variability, which can be underestimated or overestimated among species due to limited sampling size or restricted sampling distribution. Since *O. branchiatus* was the only species to show mtDNA variation, the genetic distance was plotted against geographic distance (Fig. 2) in order to investigate the issue of whether intraspecific variation could be underestimated or overestimated across both ocean basins. Although adequate representation of genetic diversity across ranges (intra-Pacific) seem apparent for Pacific specimens, the single clustering of intra-Atlantic genetic distances indicates the need for additional sampling locations in the Atlantic, possibly near the Mediterranean, equatorial region or along the eastern Atlantic realm to ensure genetic structure is not overestimated. Secondly, the noticeably high genetic distance near 0.04 in Fig. 2 for sampling sites within the same location (interregional distance) or within close geographic proximity (less than 1000 km apart) is entirely due to a single COI sequence obtained from two pooled specimens on an Atlantic *C. caretta*. Whether this is a potential outlier due to limited sampling of *O. branchiatus* from *C. caretta* was assessed by performing Mantel’s tests between genetic and geographic distances. Removal of this sampling point from the Mantel’s test analyses did not significantly impact the genetic diversity distribution. As expected, it did lower the correlation coefficient from 0.62 to 0.57, but the *p* value (< 0.01) remain unchanged. The high statistically significant positive correlation between genetic and geographic distances suggests strong isolation by distance for this species of marine leech.

Distance-based barcoding

The genetic divergences of COI sequences within the genus *Ozobranchus* was analyzed to assess the "barcoding gap" or similarity cut-off between intra- and interspecific sequences (Meyer & Pauly 2005; Meier *et al.* 2006; Meier *et al.* 2008). Since COI was conserved for *O. margoi*, the level of genetic variation within and between species of this genus was predominately influenced by the COI divergence within *O. branchiatus* (mean 1.83%, avg. SE 0.44). As expected, genetic divergence among conspecific individuals was lower than among congeneric species. K2P pairwise intraspecific divergence ranged from 0% to 4.45% with a mean of 1.58% (average standard error 0.38), while mean pairwise interspecific divergence was 18.34% (range 17.52% - 19.23%, avg. SE 1.94).

The absence of any overlap between intraspecific and interspecific divergences of COI sequences (Fig. 3) illustrates the presence of a ‘‘barcoding-" or "distance-gap'' for marine turtle leeches at this locus. Hence, the "10x rule'' threshold (15.8% in this study) proposed by Hebert *et al.* (2004) correctly identified 100% of all *Ozobranchus* spp. samples. Since the minimum congeneric K2P distance (17.52%) and maximum intraspecific distance (4.45%) are above and well below the threshold, respectively, this is strong indication both species are concordant with current taxonomy (Hebert *et al.* 2004). In addition, the 15.8% threshold suggests a similarity cut-off of around 84.2% between the two species, which corresponds with the maximum identity scores produced (range 84% - 85%) using the NCBI's nucleotide BLAST server (search results optimized under blastn).

Phylogenetics and host-parasite co-evolution

All ML trees revealed marine turtle leeches as a monophyletic group with strong bootstrap support above 95% and speciation occurring at all loci (Fig. 4). Although five mitochondrial regions have been used in sea turtle phylogenetics studies (Duchene *et al*. 2012), mtDNA control region was selected for host-parasite co-evolution assessment because it is the only mtDNA marker available for marine turtle species at geographical locations most similar in proximity to the actual turtle host locations of leech specimens analyzed. Mantel's test was performed showing a statistically significant correlation coefficient of 0.42 (p value < 0.01) between sea turtle genetic distance and the COI genetic distance of *O. branchiatus* leeches from identical sea turtle species and geographic locations.

Simple character-based barcoding

Of the 19% variable sites, 57 yielded simple pure (sPu) CAs found solely in the *Ozobranchidae* family (Appendix S7). In addition, 19 of those sites (102, 111, 123, 139, 162, 204, 291, 318, 336, 342, 369, 444, 517, 519, 546, 570, 579, 624, and 627) contain nucleotide differences responsible for distinguishing the *O. branchiatus* haplotypes (Appendix S2). Please note, positions listed in Appendix S7 are one offset lower than those given in Appendix S2 due to trimming after alignment with the alternate group.

Twelve of the diagnostic sites (15, 44, 46, 47, 55, 118, 186, 265, 353, 360, 481, and 482) have characters fixed in both species making them identifiers of this family. At the species level, *O. margoi* and *O. branchiatus* have sixteen (49, 51, 63, 69, 93, 96, 129, 138, 165, 315, 333, 336, 393, 537, 549, and 642) and eleven (24, 49, 121, 180, 240, 289, 366, 480, 484, 595, and 648) positions with CAs fixed for those given species, respectively. However, pure CAs can be limited to only certain members of the same species known as private CAs. For *O. branchiatus*, the COI barcode contains several private CAs that are also uniquely associated with certain turtle host species, a specific ocean basin, or a particular sampling location in the oceanic region. These private CAs occur at thirteen positions with one character (G) at position 519 distinguishing specimens collected from Pacific sea turtles. Leeches from eastern Pacific *L. olivacea* were the only samples that did not have diagnostic characters useful for discerning host affiliations or geographic origin. All *Ozobranchus* spp. samples from other turtles collected at different locations in the Atlantic and Pacific have at least one character identifying the specimens as specific to hosts in that area or to the location itself. It is important to note, Florida is the only site in this study with leeches taken from more than one species of sea turtle.

Identifying cocoon samples

The first study to identify *Ozobranchus* spp. cocoon samples using DNA barcoding was limited to a single posthatched sample (cocoon residue) from a northern Atlantic *C. mydas* with numerous ambiguities in the one directional sequence analyzed (McGowin *et al*. 2011). Better sequencing (two directional) results were obtained for the cocoon samples in this study with only one sample (cocoon with visible larvae) having ambiguities. This sample collected from a Hawaiian *C. mydas* had ambiguities at two locations in the 658 base pairs COI sequence (R at 163 and Y at 613) and was the only unhatched cocoon sequenced. Cocoon residues all share identical COI haplotypes with other leeches collected on the same turtle (Appendix S3). Although distance-based and character-based barcoding conclusively identified the unhatched cocoon as belonging to an *O. branchiatus* parasitizing Hawaiian *C. mydas*, haplotype designation could not be determined due to the ambiguities located at positions that differentiate haplotypes in the Hawaiian archipelago. However, incorporation of the cocoon sequence into a neighbour-joining tree (monophyly-DNA barcoding) show nearest relation to haplotype OB-A2, which matches the haplotype of the adult specimen found with the cocoon (Appendix S3).

IV. DISCUSSION

The Ozobranchidae family is notable in being the only one in the Rhynchobdellida suborder with members generally parasitic to turtle hosts rather than fishes or other aquatic invertebrates (Williams & Burreson 2006). Since it was distinguished from Piscicolidae by Richardson (1969), the Ozobranchidae family has been traditionally defined as comprising only two genera (*Bogabdella* and *Ozobranchus*) with nine accepted species (Sawyer 1986), seven alone in the *Ozobranchus* genus. A few sources have now included an additional species under a third genus (*Unoculubranchiobdella*) to the family (Christoffersen 2008) after a study by Lobo Peralta *et al*. (1998) documented *Unoculubranchiobdella expansa* as an Ozobranchidae parasite of *Podocnemis expansa* (Arrau River Turtle). This finding along with the discovery of a new Ozobranchid (*Bogabdella sp.*) on a South American turtle (*Podocnemis unifilis*) (Shain *et al*. 2007) raise the question of whether the Ozobranchidae family must be redefined once again, but in the absence of taxonomic scrutiny this possibility awaits further investigation. What is evident, however, is that *O. branchiatus* and *O. margoi* are the still the only species in the family with genetic data available and the only Ozobranchidae parasites classified as marine turtle leeches. The other remaining Ozobranchidae species are mainly associated with freshwater turtles, and except for a few publications on *Ozobranchus jantseanus* (Yamauchi & Suzuki 2008; Yamauchi *et al*. 2012), virtually no records exist of those leeches in recent years. As a result, genetic divergence comparisons were limited to the genus level and between *O. branchiatus* and *O. margoi* for this study.

Population structure and history

Morphologically, members of the small tropical Ozobranchidae family are proboscis-bearing leeches, traditionally known as rhynchobdellids. Separate phylogenetic analyses of mtDNA and nuclear ribosomal genes in this study and others show that marine turtle leeches hold an uncertain position among leeches of the paraphyletic Rhynchobdellida suborder (Sket & Tontelj 2008; Williams & Burreson 2006). However, it is evident from this study that sea turtle leeches are a monophyletic group with significant evolutionary divergence from other hirudinean clades and speciation occurring over an extensive period of time (Fig. 4).

To estimate an evolutionary time frame for the speciation event, COI substitution rates (Trajanovski *et al*. 2010; Wirchansky & Shain 2010) for other hirudinean taxa were considered in the context of *Ozobranchus* spp. evolution. Although an exact time period cannot be established due to the absence of COI molecular clock values specific to marine leeches, the observed ~16% COI sequence divergence (barcoding gap or threshold) between *O*. *branchiatus* and *O*. *margoi* suggests that speciation occurred ~16–32 mya.

Molecular clock variance values of other isthmus geminates (Knowlton & Weight 1998; Hurt *et al*. 2009) also offer a tentative estimate of when the interoceanic populations of *C*. *mydas O*. *branchiatus* separated. COI K2P sequence divergence values (Table 3) place the events of separation between the southern Atlantic populations and the nearest related Pacific population or specimens (South China Sea) to be between ~1.0-3.0 mya and between ~2.0-6.0 mya for when the northern Atlantic population separated from the nearest related Pacific population (Hawaii). It is important to note, the period of separation between the southern and northern Atlantic populations is also around ~2.0-6.0 mya with Pacific and Atlantic *C*. *mydas* divergence time reported to be 3.09 mya in Duchene *et al*. (2012). This coincides around the time of the final closure of the Isthmus of Panama (2.7-3.5 mya) (Hurt *et al*. 2009; Knowlton & Weight 1998).

Host-parasite specificity

In the case of *O. margoi*, all samples share the same single COI haplotype throughout the Atlantic and across three different turtle species, which suggests possibly either a random settlement selection by *O. margoi* on Atlantic turtle hosts or the need to continue more extensive genomic sequencing for this particular species. Potential for genetic differentiation at other loci is supported by varying levels of heterozygosity detected at 18S and 28S and the possibility of at least three histone H3 genes. Meanwhile, Mantel's test and F_{st} statistics show evidence of isolation by distance and population structure for *O. branchiatus* in both ocean basins, signifying that this species of leech is the ideal candidate for turtle-epibiont co-evolution studies.

Connections to sea turtle population ecology

Implementing effective conservation and management strategies for sea turtle species requires adequate understanding of their temporal and spatial distributions, migratory patterns, and habitat utilization (Godley *et al*. 2003; Norman *et al*. 1994). Satellite tracking, stable isotopes, and marine turtle genetics have all been utilized extensively in past studies to elucidate the migratory behavior as well as gain insights on sea turtle population biology and ecology (Duchene *et al*. 2012; Godley *et al*. 2008; Zbinden *et al*. 2011). Often overlooked are the epibionts that inhabit sea turtles.

One of the primary goals of our research is to assess whether ectoparasites, a second class of epibionts, can be used as an additional mean for understanding marine turtle evolution. Evidence from this study supports COI as an appropriate marker for exploring coevolutionary trends between sea turtles and the marine leech *O. branchiatus*. Most importantly, the shared evolutionary history can help confirm turtle population boundaries by correlating geographical distribution of the parasitic leech *O. branchiatus* with that of the host species.

For instance, satellite tracking and mtDNA data have established Hawaiian *C. mydas* turtles as being endemic to the archipelago although seldom visits by animals (mostly stranded turtles) do occur from both the eastern and western Pacific (Balazs 1976; Dutton *et al*. 2008). Similarly, COI sequencing has also indicated leeches from Hawaiian *C. mydas* as a distinct regional population. The trans-migration behavior of *L. olivacea* suggested by recent tracking data (Alfaro-Shingueto *et al*. 2011) and the occasional, albeit rare, appearance of turtles from outside rookeries in the Hawaiian archipelago offers a possible explanation to why Pacific *O. branchiatus* exhibit significantly lower nucleotide diversity compared to Atlantic *O. branchiatus* populations (Table 4).

High F_{ST} estimate reported in the Atlantic for *O. branchiatus* suggests minimal gene flow occur between the northern and southern Atlantic locations. This corresponds with studies indicating that no trans-Atlantic migration exists between *C. mydas* from Florida and Brazil (Bass & Witzell 2000; Encalada *et al*. 1996; Lahanas *et al*. 1998; Naro-Maciel *et al*. 2007; Shamblin *et al*. 2012). DNA barcoding studies using COI on marine turtles have also established a similar story where a majority of *C. mydas* from northern nesting sites were characterized by one haplotype, while those from southern or near equatorial nesting sites were fixed for a second haplotype (Naro-Maciel *et al*. 2010).

It is important to note, intra-oceanic F_{ST} estimates are higher than that between the two basins with COI and 18S analyses both showing southern Atlantic *O. branchiatus* specimens on *C. mydas* to be closer in relation to other Pacific *O. branchiatus* specimens (Fig. 1; Fig. 4; Table 3). This supports the notion that limited gene flow occurs between the two geographically separated populations. Although this could be a result of low sampling distribution in the Atlantic as illustrated in Fig. 2, there is general consensus that limited genetic exchange prohibits Atlantic and Pacific *C. mydas* populations from being considered separate species (Naro-Maciel *et al*. 2008).

Phylogeographic studies also give evidence of relatively recent linkages between Atlantic, Indian, and Pacific *C. mydas* (Roberts *et al*. 2004; Bourjea *et al*. 2007) with mitogenomic sequencing showing a shared common haplotype among *C. mydas* nesting in the southern equatorial Atlantic and southwest Indian Ocean rookeries (Bourjea *et al*. 2007; Shamblin *et al*. 2012). The haplotype relationship detected for this species of turtle combined with the phylogenetic relationships shown for the leeches in this study offer insights into a possible connection between Atlantic and Pacific *C. mydas* populations, possibly across the southern tip of Africa after the closing of the isthmus and during changes in ocean current temperature (Duchene *et al*. 2012).

Distance versus diagnostics

All approaches to DNA barcoding rely on the availability of genetic data of related taxa with nearest phylogenetic relations (congeneric species) and thorough morphological assessment. Consequently, DNA barcoding is a much less effective species identification tool for taxa with limited taxonomic scrutiny (Meyer & Paulay 2005). These limitations to DNA barcoding are most prominent in the distance-based approach and well illustrated in the case of the Ozobranchidae family.

The absence of genetic information from freshwater turtle leeches in the *Ozobranchus* genus and other genera increases the potential of overestimating the "10x rule" threshold, which in this study (16%), is significantly higher than the proposed cut-off for birds and turtles (2%)(Herbert et al. 2004; Reid *et al*. 2011), although it should be noted that vertebrates seem to have a lower reported threshold than invertebrates, such as gastropods (6.4% and 11.9%) (Zou *et al*. 2011; Zou *et al*. 2012). It is important to also consider the fact this is a very small family with the targeted taxa being restricted to only two species in a single genus. The small taxa size coupled with significant interspecific divergence between the two sister species marginalize the impact that missing congeneric distance might have on assessing the barcoding gap for marine turtle leeches. Thus, the 16% threshold reported in this study may not be the definitive threshold for Ozobranchidae annelids, but more than likely, it is an accurate estimation of the threshold for identifying marine turtle leeches around the world.

The primary disadvantage of not having genetic data from other freshwater Ozobranchids is the inability to assess a minimum congeneric distance, which is necessary in order to properly flag cryptic species diversity within the global *O. branchiatus* population (Hebert *et al*. 2004). Furthermore, the absence of *O. margoi* samples from the Pacific can lead to underestimating the level of genetic diversity for this species. Although GenBank sequences were available from other studies for *O. margoi* besides McGowin *et al*. (2011), the available data (obtained from a single specimen) was not utilized due to numerous gaps inconsistent with results from our study. Inconsistency in GenBank-archived sequences has been reported as high as 49% with over 70% of which can be attributed to field- or laboratory-based error (Williams *et al*. 2013). These anomalies in GenBank sequences are another contributing factor to the difficulties of establishing an accurate barcoding threshold.

The problematic issues hampering the distance-based method are more easily resolved with character-based DNA barcoding. Unlike the traditional distanced-based approach, the character-based technique is not dependent on the accurate establishment of a single interspecific threshold for a given taxa. Its success lies strictly in the existence of diagnostic sites that differentiate separate species. Thus, even if there is a loss of CAs in the COI barcode (Appendix S7) from future incorporation of other freshwater Ozobranchidae species, accurate species identification at all life stages is still possible if adequate number of diagnostic sites remain in the barcode, making this approach more suited for identifying less studied taxa, such as the Ozobranchidae family.

McGowin *et al*. (2011) has shown that even when numerous ambiguities occur in a sequenced sample, character-based DNA barcoding can still accurately identify the specimen if enough CAs are available at the given locus. Although no criteria has been establish on what is the minimal number of CAs a barcode must have to be effective, it is apparent in this study that relying on a single unique CA is troublesome due to the possibility of ambiguities occurring at informative sites. Thus, species identification dependant on limited simple CAs or diagnostic sites is vulnerable to the same problems as the distanced-based approach. However, this issue can be resolved with the utilization of compound character-based DNA barcoding for the acquisition of more needed CAs. The same cannot be said regarding the distance-based method, which requires complete taxonomic sampling as the only option to accurately estimate the DNA barcoding threshold. In view of the difficulties associated with sampling (rarity of specimens or host species, politically inaccessible locations, etc.), this may not be a feasible option, especially for understudied taxa.

Novel applications for DNA barcoding

Evidence that character-based DNA barcoding can assign ocean basin origin for sea turtles was first given in Naro-Maciel *et al*. (2010). Results from this study strongly support that character-based DNA barcoding can also assign ocean basin origin for turtle epibionts and possibly the specific location the ectoparasite originated. Although no diagnostic CAs were obtained for western Pacific leeches from Mexican *L. olivacea*, tree-based barcoding methods can efficiently discern species identity and geographic location of those specimens (Fig. 4a; Appendix S3). Monophyly-barcoding is also an effective alternative approach when missing information arises at informative sites, such as in the case with the Hawaiian cocoon sample (Appendix S3). Furthermore, distanced-based (NJ) and character-based (ML) COI trees share identical topology for marine Ozobranchidae leeches with respect to the same outgroup (Fig. 4a; Appendix S3), suggesting that distance or diagnostics tree-based methods are equally effective for distinguishing these two species and retrieving geographic information.

Although a sufficient number of simple pure diagnostic sites were found to differentiate *O. margoi* from *O. branchiatus*, utilization of compound DNA barcoding can enhance that number, which is especially needed in cases where ocean basin assignment is limited to only a few or single pure character position. Compound character DNA barcoding is a relatively under-used method, but when employed, it has been shown to be effective in differentiating species that yield inadequate number of simple CAs (Lowenstein *et al.* 2009; Ludington *et al.* 2012). Compound character analysis can be implemented in the program Character Attribute Organization System (CAOS)(Sarkar *et al.* 2008). Two previous case studies using CAOS suggest this method can be susceptible to error (Kerr *et al.* 2009) and not efficient when applied on taxonomically challenging groups, such as polyphyletic species (Yassin *et al.* 2010). If additional diagnostic characters cannot be obtained from compound DNA barcoding, future studies will need to target other mtDNA loci to acquire more simple pure CAs.

Conclusions

Even though the distance-based method is still the gold standard for DNA barcoding, it is a much less desirable option for species identification in the case of understudied taxa with incomplete taxonomic sampling. In the case of the Ozobranchidae family, these issues can be adequately resolved with the character-based approach. Furthermore, with the advent of next generation sequencing, which offers rapid generation of data at high volume (Taylor & Harris

2012), the future and advancement of DNA barcoding as a successful tool in species conservation and management must extend beyond the obvious utility of species identification. To achieve this, current DNA barcoding studies must incorporate novel techniques or capitalize on new potential applications. Our study present evidence that DNA barcoding can serve as a convenient tool for determining the geographical location of *O. branchiatus* specimens at all stages of development. Being able to correlate epibiont with ocean basin origin will offer meaningful insights on marine turtle population ecology. Most of all, the shared evolutionary history of ectoparasite and host will help discern the migration patterns and population boundaries of threatened sea turtle populations.

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Table 1

PCR primers used in amplification and sequencing.

Name	Sequence 5'-3'	Source
COI		
LCO1490	GGTCAACAAATCATAAAGATATTGG	(Folmer *et al.* 1994)
HCO2198	*TAAACTTCAGGGTGACCAAAAAATCA*	(Folmer *et al.* 1994)
28S		
C1'	ACCCGCTGAATTTAAGCAT	(Lê *et al.* 1993)
C2	*TGAACTCTCTCTTCAAAGTTCTTTTC*	(Lê *et al.* 1993)
18S		
1F	TACCTGGTTGATCCTGCCAGTAG	(Giribet *et al.* 1996)
5R	*CTTGGCAAATGCTTTCGC*	(Giribet *et al.* 1996)
3F	GTTCGATTCCGGAGAGGGA	(Giribet *et al.* 1996)
18Sbi	*GAGTCTCGTTCGTTATCGGA*	(Giribet *et al.* 1996)
18Sa2.0	ATGGTTGCAAAGCTGAAAC	(Giribet *et al.* 1996)
9R	*GATCCTTCCGCAGGTTCACCTAC*	(Giribet *et al.* 1996)
Histone H3		
H3af	ATGGCTCGTACCAAGCAGACVGC	(Colgan *et al.* 1998)
H3R1	*CCAACCAAGTACGCCTCA*	(Lavretsky *et al.* 2012)
H3R2	*CCAACCAAGTAAGCCTCG*	(Lavretsky *et al.* 2012)

Italics: reverse primers

Table 2 Nucleotide substitution pattern, nucleotide frequencies, and nucleotide and amino acid variability as estimated in MEGA 5. Transitions rates are in bold, while transversion rates are italicized. OM GenBank not used due to not being 658 bp.

Maximum composite likelihood estimate of substitution pattern

	A	T	C	G
A	-	*2.73*	*1.2*	**16.31**
T	*2.25*	-	**11.52**	*1.17*
C	*2.25*	**26.24**	-	*1.17*
G	**31.23**	*2.73*	*1.2*	-

Nucleotide frequencies

A	0.306
T	0.372
C	0.163
G	0.160

Proportion of sites variable

	Variable	Total	% Variable
Nucleotide	126	658	19
Amino acid	5	219	2

Table 3 Host and geographical associations for the ten COI haplotypes of *O. branchiatus* identified in this study or obtained from GenBank. On the diagonal are the total number of positions with simple private (sPr) characters (Table 5) unique only to that specific haplotype. Average pairwise divergences between haplotypes calculated using the Kimura 2-parameter model (K2P) are above the diagonal. Below the diagonal are the numbers of mutated positions (substitutions) between the haplotypes. How many of those substitutions contain sPr characteristic attributes is bolded and given in parentheses.

Host-Site (n, ht)	COI haplotype (sq, *)	OB-A1	OB-A2	OB-A3	OB-A4	OB-P1	OB-P2	OB-P3	OB-P4	OB-P5	OB-P6
CM-FL (37, 14)	OB-A1 (19, 7)	**0**	0.040	0.002	0.030	0.030	0.031	0.031	0.028	0.027	0.031
CC-FL (2, 1)	OB-A2 (1, 1)	25 (**12**)	**6**	0.041	0.043	0.043	0.044	0.041	0.038	0.040	0.041
CM-FL (1, 1)	OB-A3 (1)	1	26 (**12**)	**0**	0.031	0.031	0.033	0.033	0.030	0.028	0.033
CM-BZ (6, 5)	OB-A4 (6)	19 (**8**)	27 (**12**)	20 (**8**)	**1**	0.017	0.019	0.019	0.019	0.017	0.014
CM-HI (30, 13)	OB-P1 (17, 4)	19 (**10**)	28 (**14**)	20 (**10**)	11 (**5**)	**0**	0.002	0.002	0.008	0.006	0.012
CM-HI (2, 2)	OB-P2 (2)	20 (**10**)	28 (**14**)	21 (**10**)	12 (**5**)	1	**0**	0.003	0.009	0.008	0.014
CM-HI (2, 2)	OB-P3 (2)	20 (**11**)	26 (**12**)	21 (**11**)	12 (**5**)	1	2 (**1**)	**0**	0.009	0.008	0.011
LO-MX (1, 1)	OB-P4 (1)	18 (**9**)	24 (**11**)	19 (**9**)	12 (**4**)	5 (**2**)	6 (**2**)	6 (**3**)	**0**	0.002	0.014
LO-MX (1, 1)	OB-P5 (1)	17 (**9**)	25 (**12**)	18 (**9**)	11 (**4**)	4 (**1**)	5 (**1**)	5 (**2**)	1 (**1**)	**0**	0.012
CM-CHI (2, 1)	OB-P6 (2)	20 (**10**)	26 (**11**)	21 (**10**)	9 (**4**)	8 (**5**)	9 (**5**)	7 (**4**)	9 (**5**)	8 (**4**)	**1**

Abbreviations

Number of specimens collected (n) and number of turtle hosts (ht) including those from McGowin *et al*. 2011. Total number of COI sequences matching that haplotype (sq) Number of matching sequences from pooled samples (*)	Sea turtle species: *C. mydas* (CM), *C. caretta* (CC), *E. imbricata* (EI), *L. olivacea* (LO) Geographical Locations/Sites: Barbados (BB), Brazil (BZ), Florida (FL), Hawaii (HI), Hong Kong/South China Sea (CHI), Mexico (MX)

Table 4 DNA sequence diversity of *Ozobranchus branchiatus* leeches: number of sequences including those from pooled samples (bolded and italicized), haplotype diversity (H), nucleotide diversity (π), and their standard deviations for COI and 18S. All genetic data obtained from this study and McGowin *et al*. 2011.

Locus		H	π
COI (***52***)		0.755 ± 0.039	0.01779 ± 0.00104
	Atlantic (***27***)	0.470 ± 0.096	0.01285 ± 0.00309
	Pacific (***25***)	0.537 ± 0.115	0.00326 ± 0.00113
18S (***15***)		0.590 ± 0.106	0.00051 ± 0.00009
	Atlantic (***6***)	0.600 ± 0.215	0.00047 ± 0.00019
	Pacific (***9***)	0.222 ± 0.166	0.00012 ± 0.00009

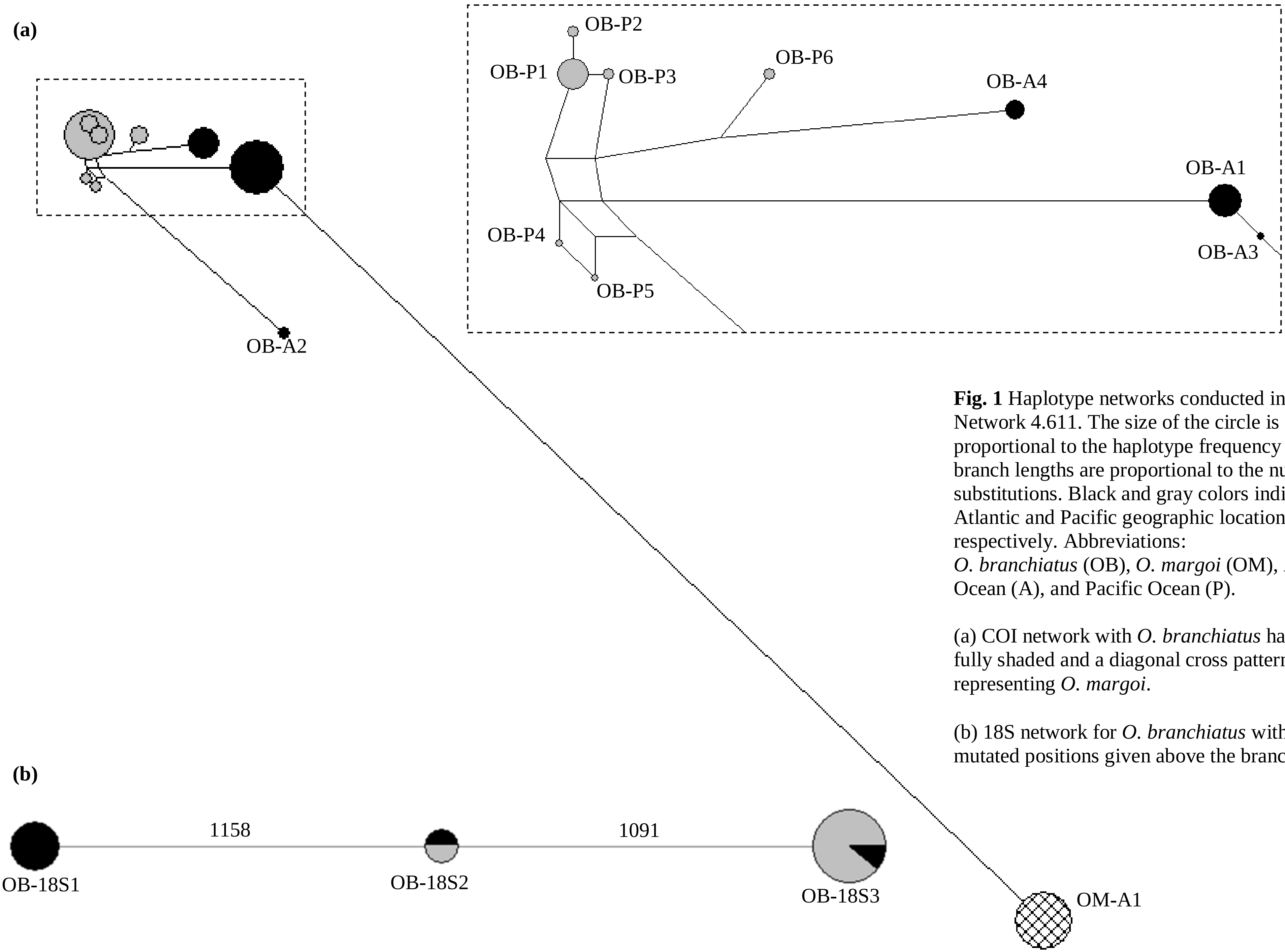


Fig. 1 Haplotype networks conducted in Network 4.611. The size of the circle is proportional to the haplotype frequency and branch lengths are proportional to the number of substitutions. Black and gray colors indicate Atlantic and Pacific geographic locations, respectively. Abbreviations: *O. branchiatus* (OB), *O. margoi* (OM), Atlantic Ocean (A), and Pacific Ocean (P).

(a) COI network with *O. branchiatus* haplotypes fully shaded and a diagonal cross pattern representing *O. margoi*.

(b) 18S network for *O. branchiatus* with mutated positions given above the branch length.

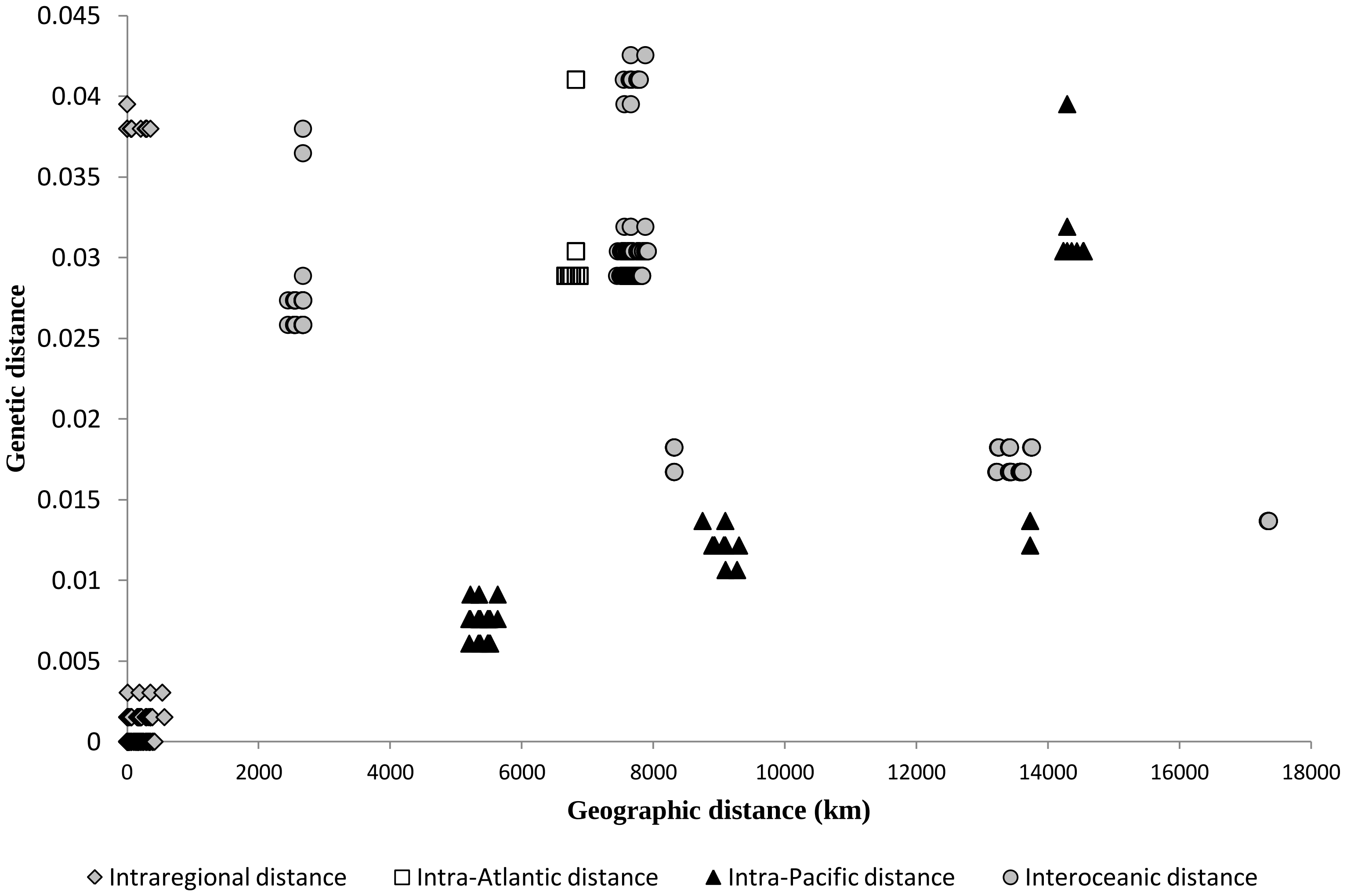


Fig. 2 The geographic distance between where *O. branchiatus* specimens were collected is plotted against their COI divergence. Intraregional distance implies distance between sampling sites within the same geographic location or proximity (e.g., Indian River Lagoon, Florida and Hutchinson Island, Florida). Intra-Atlantic or Intra-Pacific denotes distances between different locations confine to the Atlantic or Pacific basin, respectively. All genetic distance was computed by Alleles in Space version 1.0 with 1000 permutations.

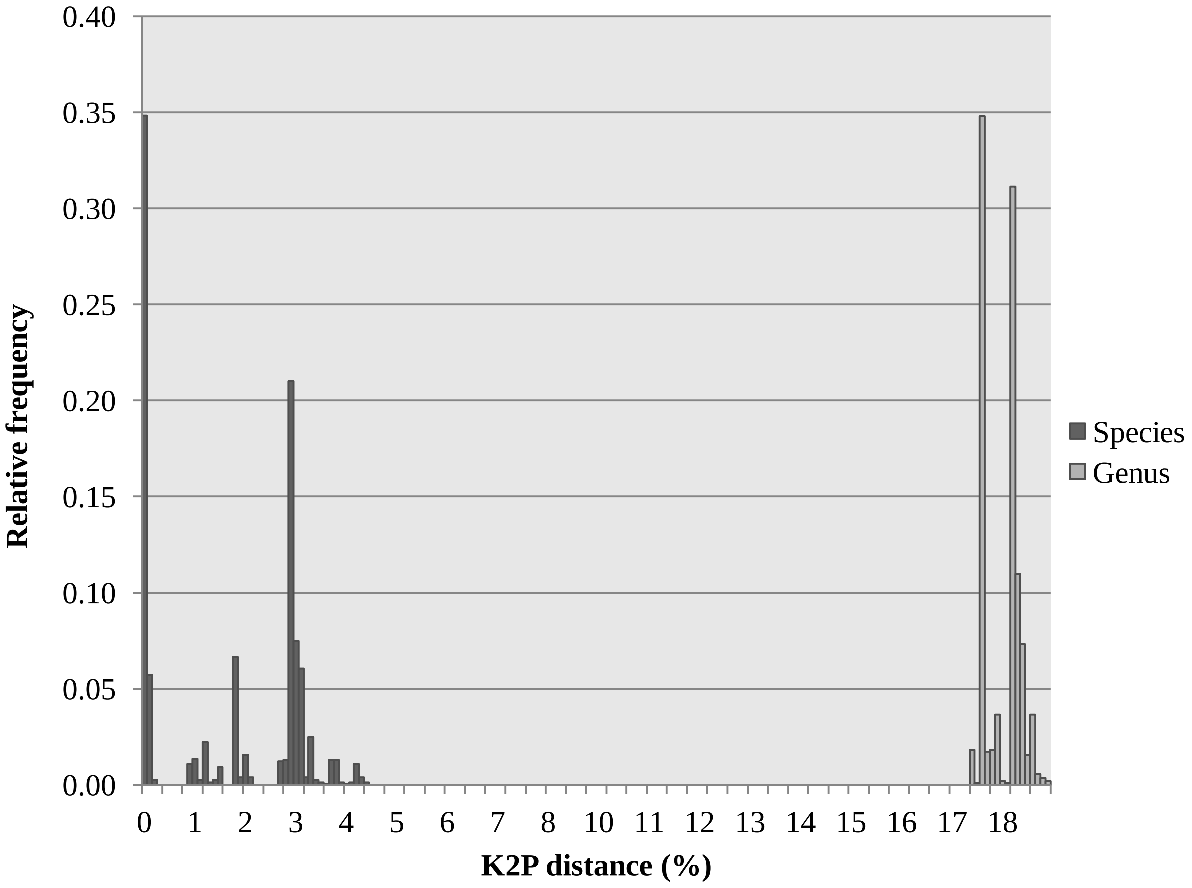


Fig. 3 Distribution of genetic divergences based on the K2P distance model for COI sequences.

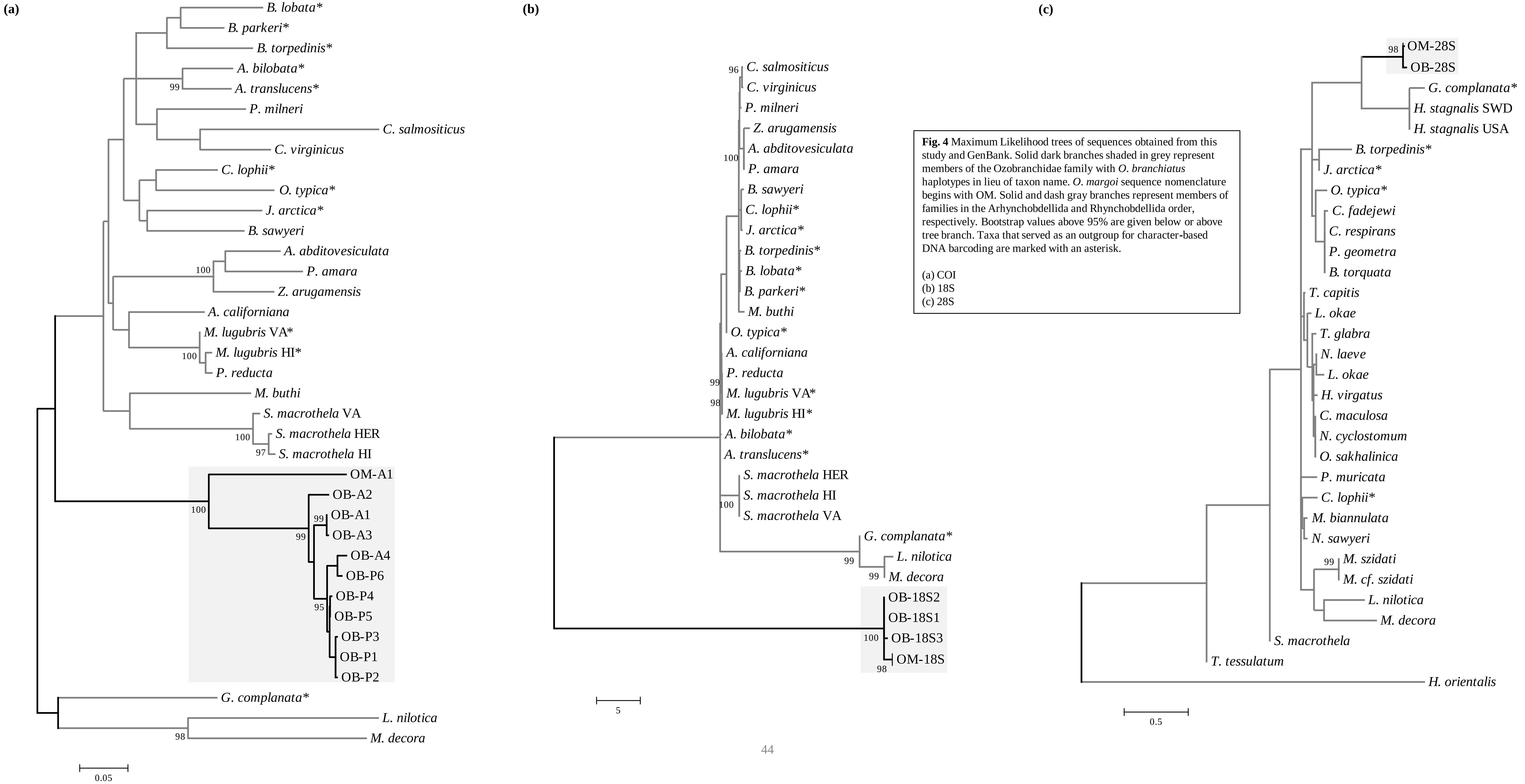


Fig. 4 Maximum Likelihood trees of sequences obtained from this study and GenBank. Solid dark branches shaded in grey represent members of the Ozobranchidae family with *O. branchiatus* haplotypes in lieu of taxon name. *O. margoi* sequence nomenclature begins with OM. Solid and dash gray branches represent members of families in the Arhynchobdellida and Rhynchobdellida order, respectively. Bootstrap values above 95% are given below or above tree branch. Taxa that served as an outgroup for character-based DNA barcoding are marked with an asterisk.

(a) COI
(b) 18S
(c) 28S

Higher Taxon	Turtle host (ht)	Source	GPS	Collection Date	x	y	z	Genbank Accession Numbers		
								COI	18S	28S
Rhynchobdellida Ozobranchidae *Ozobranchus branchiatus*		**Florida, USA**								
	Caretta caretta (1)	Hutchinson Island	27.345, -80.240278	8/25/2009	2	1	2	GU985466†§	KF728215‡§	KF728224‡§
	Chelonia mydas (15)	Hutchinson Island	27.345, -80.240278	3/21/2010	3	1	2	GU985465†§; OB-A1‡	KF728214‡§	KF728224‡§
			27.345, -80.240278	3/23/2010**	2	1	2	GU985465†§		
		Hutchinson Island (Cold stun event)	27.345, -80.240278	3/11/2010	1			KF728206‡		
		Indian River Lagoon	27.8325, -80.438333	8/12/2009	3	1	3	OB-A1‡§		
			27.8325, -80.438333	12/07/2009	12	2	6, 6	GU985465†§	KF728214‡§	KF728224‡§
			27.8325, -80.438333	12/07/2009	1			OB-A1†		
			27.8325, -80.438333	11/30/2009	1			OB-A1‡		
			27.8325, -80.438333	11/30/2009	1			OB-A1‡		
		Grassy Key mm 57, Key West		11/14/2010	3	1	2	OB-A1‡£	KF728214‡£	KF728224‡§
		Barracouta, Key West		5/10/2010	4	1	4	OB-A1‡§		
		Cape Sable		3/21/2011	1			OB-A1‡		
		Long Key State Park		3/28/2011	1			OB-A1‡		

	Monroe County Long Key Lake		6/11/2011	1			OB-A1‡		
	Palm Beach County		10/14/2011	2			OB-A1‡		
	Key West	24.754673, -81.023805	2/09/2011	2			OB-A1‡		
	Espirito Santo, Brazil								
Chelonia mydas (5)	Curva da Jurema, Vitoria	-20.30812, -40.29073	7/15/2011	1			KF728207‡		
	Praia da Iate Clube, Vitoria	-20.30088, -40.29092	7/28/2011*	1			KF728207‡	KF728216‡	
	Prainha, Vila Velha	-20.19475, -40.17744	8/03/2011	2			KF728207‡		
	Camburi, Vitoria	-20.28802, -40.29042	9/28/2011	1			KF728207‡		
	Ilha do Frade, Vitoria		11/14/2011	1			KF728207‡		
	Hawaii, USA								
Chelonia mydas (17)	Kahana Beach Park, Oahu	21.55595, -157.87263	9/24/2010	6	1	5	KF728208‡£		
	West Loch, Oahu	21.3488, -157.9881	11/18/2010	5	1	4	KF728208‡£	KF728216‡§	KF728224‡§
	Kaneohe Bay, Oahu	21.46570, -157.84330	7/23/2011	1			KF728208‡		
		21.46252, -157.83283	4/25/2011	1			KF728208‡	KF728216‡	
		21.49308, -157.84739	5/25/2011	1			KF728208‡		
	Waialua, Oahu	21.58281, -158.14382	12/30/2010	1			KF728208‡		

	Wainae Boat Harbor, Oahu	21.450411, -158.19776	1/06/2011	1			KF728208‡		
	Waianae (Pililaau Army Center), Oahu	21.44579, -158.18954	2/19/2011	1			KF728208‡		
	Lagoon Drive, Oahu	21.31213, -157.91978	4/10/2011	1			KF728208‡		
	Kalapolepo Fish Pond, Maui	20.7774, -156.4597	11/15/2010	5	1	4	KF728208‡£	KF728216‡§	KF728224‡§
	Lahaina, Maui	20.846875, -156.65536	11/28/2010	5	1	4	KF728208‡£	KF728216‡§	KF728224‡§
	Kihei (Malama St), Maui	20.7357, -156.4562	1/26/2011	1			KF728209‡		
	Makena (Palauea Beach), Maui	20.673591, -156.44374	3/10/2011	1			KF728210‡		
	Ho’okipa Beach Park, Maui	20.9336, -156.3574	11/23/2011	1			KF728208‡		
	Anini Beach, Kauai	22.216447, -159.42914	12/05/2011	1			KF728209‡	KF728216‡	
	Keaukaha (Hilo), Hawaii	19.7331, -155.0241	2/08/2011	1			KF728210‡	KF728216‡	
	Kapoho, Hawaii	19.4983, -154.8197	11/20/2011	1			KF728208‡		
	Jalisco State, Mexico								
Lepidochelys olivacea (2)	Campamento la Gloria		8/08/2008	1			KF728212‡	KF728216‡	
	Costa Careyes, Playa Ventanas		7/26/2008	1			KF728211‡	KF728216‡	
	South China Sea								
Chelonia mydas (1)	Hong Kong	22.191558, 114.136664	8/14/2012	2			KF728213‡	KF728215‡	

Ozobranchus margoi		**Barbados**								
	Eretmochelys imbricata (5)	Needham’s Point		7/21/2011	1			OM-A1‡	KF728220‡	KF728227‡
		Needham’s Point		7/19/2011	1			OM-A1‡	KF728217‡	KF728227‡
		Needham’s Point		8/01/2011	1			OM-A1‡	KF728221‡	KF728227‡
		Needham’s Point		8/29/2011	1			OM-A1‡	KF728219‡	KF728226‡
		Needham’s Point		7/13/2011	1			OM-A1‡	KF728222‡	KF728226‡
		Florida, USA								
	Caretta caretta (6)	St. Johns County Intercoastal		8/4/2010	1			OM-A1‡		
		Daytona Beach		4/14/2010	1			OM-A1‡	KF728219‡	KF728226‡
		Ponce Inlet		5/11/2010	4	1	3	OM-A1‡£	KF728217‡	KF728226‡
		Hutchinson Island	27.345, -80.240278	8/13/2009	2	1	2	GU985467†§		
			27.345, -80.240278	3/24/2010	2	1	2	OM-A1‡§	KF728218‡§	KF728225‡§
			27.345, -80.240278	9/15/2009	1			OM-A1‡		
	Chelonia mydas (3)	Hawks Channel		5/22/2011	1			OM-A1‡	KF728217‡¥	KF728226‡¥
		Vero Beach		7/03/2010	3	1	2	OM-A1‡£		
		Hutchinson Island	27.345, -80.240278	3/23/2010**	1			HM590711†	KF728217‡¥	KF728226‡¥

	Espirito Santo, Brazil					
Chelonia mydas (4)	Curva da Jurema, Vitoria	-20.30685, -40.29053	7/15/2011	1	OM-A1‡	
		-20.30088, -40.29092	9/28/2011	1	OM-A1‡	
	Praia da Iate Clube, Vitoria	-20.30088, -40.29092	7/28/2011*	1	OM-A1‡	KF728223‡
	Aracruz, Santa Cruz	-19.95519, -40.14027	11/03/2011	1	OM-A1‡	

Appendix S1 Descriptive data for all *Ozobranchus* spp. taxa and sequences included in this study. Major geographic locations (bolded) are listed along with sea turtle sampling sites (not bolded) and onsite GPS coordinates. Species of sea turtle host sampled at each location provided along with the total number of different turtle hosts sampled (ht) for each given species. The number of specimens collected (x), number of pooled samples (y), and number of specimens pooled per sample (z) are also given. Single or double red asterisks indicate collection dates are identical because leech specimens were collected from the same individual sea turtle. Accession number designated with a ′§′ indicates GenBank sequence was obtained from pooled samples. If individual specimens and pooled samples from the same individual turtle yield identical sequences, then the accession number is designated with a ′£′. ′¥′ indicates one-directional sequencing. Haplotype designations are given in lieu of accession numbers if genetic data match what is already in GenBank. References: This study (‡); McGowin *et al*. 2011 (†).

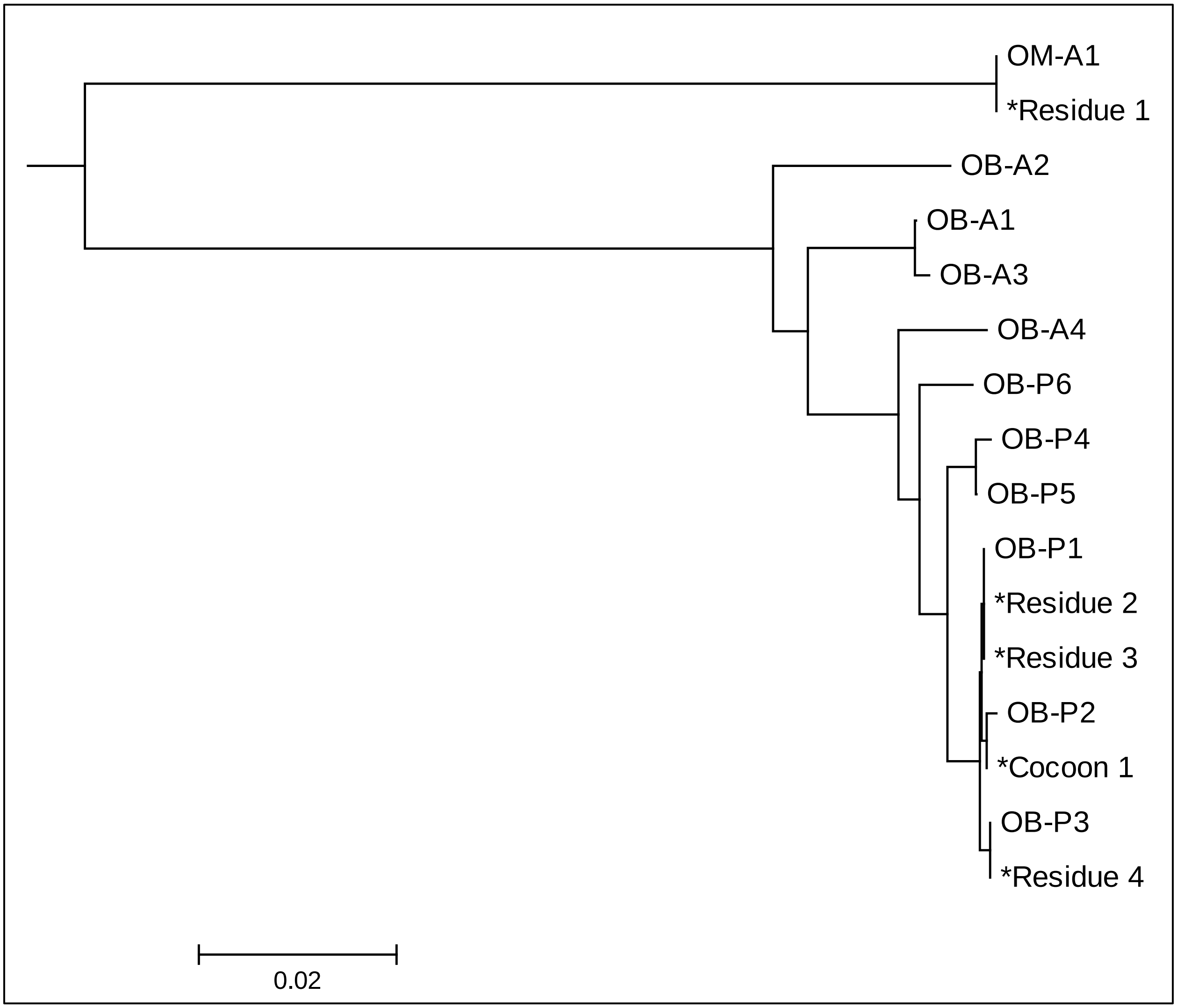
Appendix S2 Spreadsheet with information supplementing Table 3. Listed below the diagonal are all mutated positions (substitutions) between the haplotypes with substitutions containing sPr characteristic attributes bolded.

COI haplotype	OB-A1	OB-A2	OB-A3	OB-A4	OB-P1	OB-P2	OB-P3	OB-P4	OB-P5	OB-P6
OB-A1	**0**	0.040	0.002	0.030	0.030	0.031	0.031	0.028	0.027	0.031
OB-A2	**103**, **140**, **163**, 199, 202, 283, **292**, 305, **319**, 322, **343**, 347, **370**, 424, 466, 496, **518**, **535**, 553, **571**, 586, 607, **625**, **628**, 652	**6**	0.041	0.043	0.043	0.044	0.041	0.038	0.040	0.041
OB-A3	610	**103**, **140**, **163**, 199, 202, 283, **292**, 305, **319**, 322, **343**, 347, **370**, 424, 466, 496, **518**, **535**, 553, **571**, 586, 607, 610, **625**, **628**, 652	**0**	0.031	0.031	0.033	0.033	0.030	0.028	0.033
OB-A4	**112**, **124**, 283, **292**, 322, 418, 466, 511, **518**, 533, 542, **547**, 553, **571**, **580**, 607, **628**, 646, 653	**103**, **112**, **124**, **140**, **163**, 199, 202, 305, **319**, **343**, 347, **370**, 418, 424, 496, 511, 533, 535, 542, **547**, **580**, 586, **625**, **628**, 646, 652, 653	**112**, **124**, 283, **292**, 322, 418, 466, 511, **518**, 533, 542, **547**, 553, **571**, **580**, 607, 610, **628**, 646, 653	**1**	0.017	0.019	0.019	0.019	0.017	0.014
OB-P1	**112**, **124**, **205**, 235, 283, **292**, 322, 412, **445**, 466, **518**, **520**, 553, **571**, **580**, 607, **628**, 646, 653	**103**, **112**, **124**, **140**, **163**, 199, 202, **205**, 235, 305, **319**, **343**, 347, **370**, 412, 424, **445**, 496, **520**, 535, **580**, 586, **625**, **628**, 646, 652, 653	**112**, **124**, **205**, 235, 283, **292**, 322, 412, **445**, 466, **518**, **520**, 553, **571**, **580**, 607, 610, **628**, 646, 653	**112**, **205**, 235, 412, 418, **445**, 511, **520**, 533, 542, **547**	**0**	0.002	0.002	0.008	0.006	0.012

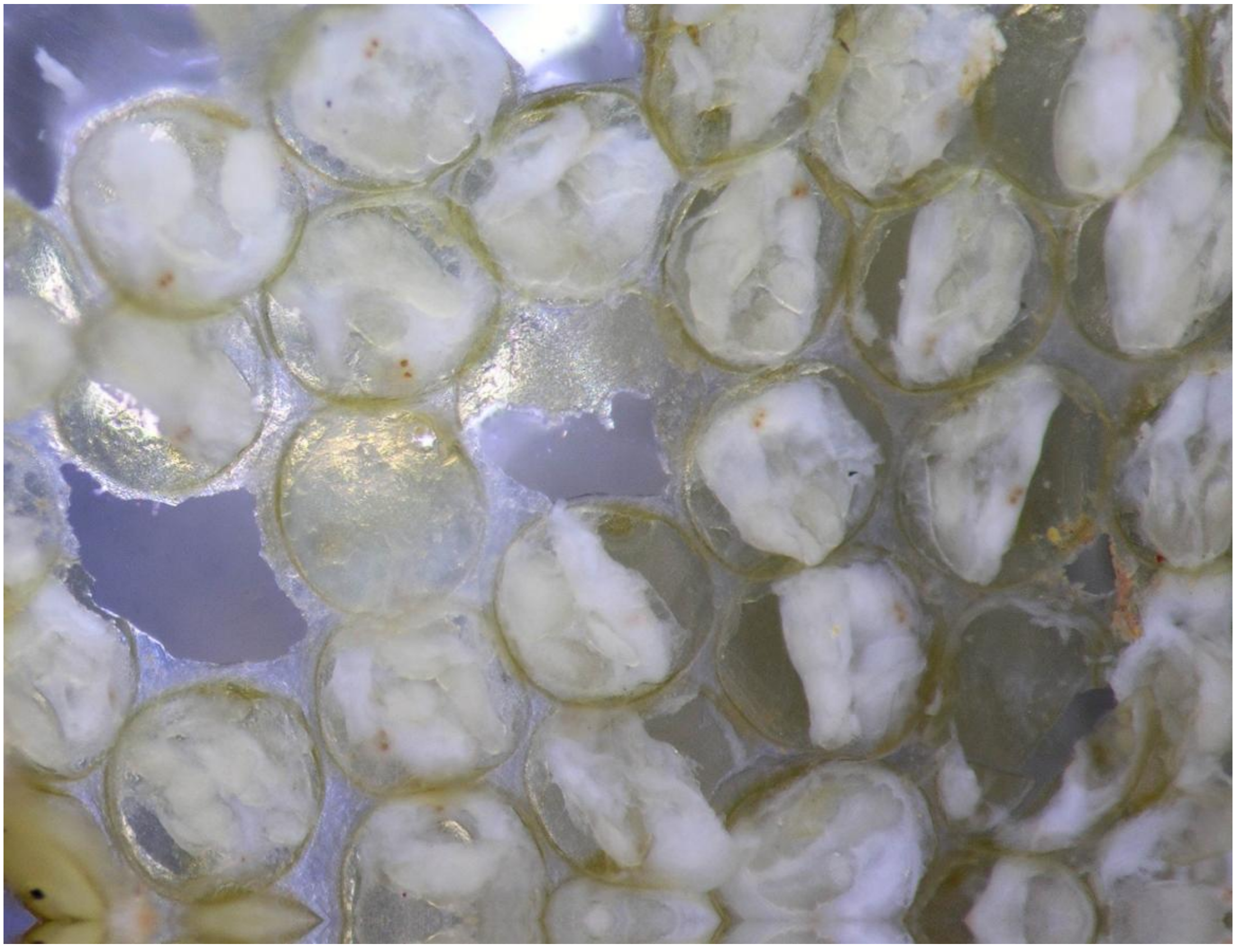
OB-P2	**112**, **124**, **205**, 235, 283, **292**, 322, 412, **445**, 466, **518**, **520**, 553, **571**, **580**, 607, 613, **628**, 646, 653	**103**, **112**, **124**, **140**, **163**, 199, 202, **205**, 235, 305, **319**, **343**, 347, **370**, 412, 424, **445**, 496, **520**, 535, **580**, 586, 613, **625**, **628**, 646, 652, 653	**112**, **124**, **205**, 235, 283, **292**, 322, 412, **445**, 466, **518**, **520**, 553, **571**, **580**, 607, 610, 613, **628**, 646, 653	**112**, **205**, 235, 412, 418, **445**, 511, **520**, 533, 542, **547**, 613	613	**0**	0.003	0.009	0.008	0.014
OB-P3	**112**, **124**, **163**, **205**, 235, 283, **292**, 322, 412, **445**, 466, **518**, **520**, 553, **571**, **580**, 607, **628**, 646, 653	26, **112**, **124**, **140**, 199, 202, **205**, 235, 305, **319**, **343**, 347, **370**, 412, 424, **445**, 496, **520**, 535, **580**, 586, **625**, **628**, 646, 652, 653	**112**, **124**, **163**, **205**, 235, 283, **292**, 322, 412, **445**, 466, **518**, **520**, 553, **571**, **580**, 607, 610, **628**, 646, 653	**112**, 163, **205**, 235, 412, 418, **445**, 511, **520**, 533, 542, **547**	613	**163**, 613	**0**	0.009	0.008	0.011
OB-P4	**112**, **124**, 140, 235, 283, **292**, 322, **445**, 466, **518**, **520**, 553, **571**, **580**, 607, **628**, 653	103, **112**, **124**, **163**, 199, 202, 235, 305, **319**, **343**, 347, **370**, 424, **445**, 496, **520**, 535, **580**, 586, 598, **625**, **628**, 652, 653	**112**, **124**, 140, 235, 283, **292**, 322, **445**, 466, **518**, **520**, 553, **571**, **580**, 598, 607, 610, **628**, 653	**112**, 140, 235, 418, **445**, 511, **520**, 533, 542, **547**, 598, 646	**140**, **205**, 412, 598, 646	**140**, **205**, 412, 598, 613, 646	**140**, **163**, **205**, 412, 598, 646	**0**	0.002	0.014
OB-P5	**112**, **124**, 235, 283, **292**, 322, **445**, 466, **518**, **520**, 553, **571**, **580**, 598, 607, **628**, 653	103, **112**, **124**, **140**, **163**, 199, 202, 235, 305, **319**, **343**, 347, **370**, 424, **445**, 496, **520**, 535, **580**, 586, 598, **625**, **628**, 652, 653	**112**, **124**, 235, 283, **292**, 322, **445**, 466, **518**, **520**, 553, **571**, **580**, 598, 607, 610, **628**, 653	**112**, 235, 418, **445**, 511, **520**, 533, 542, **547**, 598, 646	**205**, 412, 598, 646	**205**, 412, 598, 613, 646	**163**, **205**, 412, 598, 646	**140**	**0**	0.012
OB-P6	91, **112**, **124**, **163**, 235, 283, **292**, 322, **337**, 466, **518**, **520**, 533, 553, **571**, **580**, 607, **628**, 646, 653	91, 103, **112**, **124**, **140**, 199, 202, 235, 305, **319**, **337**, **343**, 347, **370**, 424, 496, **520**, 533, 535, **580**, 586, **625**, **628**, 646, 652, 653	91, **112**, **124**, **163**, 235, 283, **292**, 322, **337**, 466, **518**, **520**, 533, 553, **571**, **580**, 607, 610, **628**, 646, 653	91, **163**, 235, **337**, 418, 511, **520**, 542, **547**	91, **112**, **163**, **205**, **337**, 412, **445**, 533	91, **112**, **163**, **205**, **337**, 412, **445**, 533, 613	91, **112**, **205**, **337**, 412, **445**, 533	91, **112**, **140**, **163**, **337**, **445**, 533, 598, 646	91, **112**, **163**, **337**, **445**, 533, 598, 646	**1**

Appendix S3 Host and geographical associations for all morphologically indistinguishable samples sequenced in this study. Samples include specimens at the cocoon stage and cocoon residues (posthatched samples). The identity of these samples is provided along with the samples' matching COI haplotype.

Cocoon Sample	Turtle host	Source	*Ozobranchus* spp.	Haplotype
Residue 1	*Caretta caretta*	St. Lucie Power Plant on Hutchinson Island, Florida	*Ozobranchus margoi*	OM-A1
Residue 2	*Chelonia mydas*	Kahana Beach Park, Oahu	*Ozobranchus branchiatus*	OB-P1
Residue 3	*Chelonia mydas*	Wainae Boat Harbor, Oahu	*Ozobranchus branchiatus*	OB-P1
Cocoon 1	*Chelonia mydas*	Anini Beach, Kauai	*Ozobranchus branchiatus*	OB-P2
Residue 4	*Chelonia mydas*	Keaukaha (Hilo), Hawaii	*Ozobranchus branchiatus*	OB-P3



Neighbor-joining tree generated in MEGA5 for COI. All *O. branchiatus* haplotypes in this study were used in the analyses to assign haplotype designation for the morphologically indistinguishable samples (*) listed in the previous Table.



Cocoon collected from a *Chelonia mydas* at Anini Beach, Kauai. Leech specimens were still visible in the sample and were genetically identified as *Ozobranchus branchiatus*. Photos stacked using CombineZ and enhanced with Adobe Photoshop. Nikon Microscope Camera. Photo Credit: Triet M. Truong

Higher Taxon	**Family (*subfamily)**	**Genus**	**Species**	**Locality**	**References (COI;18S)**	**Genbank Accession Numbers**	
						COI	18S
Rhynchobdellida							
	Glossiphoniidae						
		Glossiphonia	*complanata*	England	Light & Siddall 1999; Apakupakul *et al.* 1999	AY047321	AF115982
	Piscicolidae						
	Piscicolinae*						
		Branchellion	*lobata*	California, USA	Williams & Burreson 2006	DQ414307	DQ414261
			parkeri	Tasmania, Australia	Williams & Burreson 2006	DQ414308	DQ414262
			torpedinis	South Carolina, USA	Siddall & Burreson 1998; Apakupakul *et al.* 1999	AF003265	AF115993
		Calliobdella	*lophii*	Norway	Williams & Burreson 2006	DQ414314	DQ414268
		Cystobranchus	*salmositicus*	British Columbia, Canada	Williams & Burreson 2006	DQ414316	DQ414270
			virginicus	North Carolina, USA	Williams & Burreson 2006	DQ414317	DQ414271
		Johanssonia	*arctica*	Newfoundland, Canada	Williams & Burreson 2006	DQ414320	DQ414274
		Piscicola	*milneri*	Quebec, Canada	Williams & Burreson 2006	DQ414337	DQ414292
	Platybdellinae*						
		Aestabdella	*adbitovesiculata*	Hawaii, USA	Williams & Burreson 2006	DQ414300	DQ414254
		Austrobdella	*bilobata*	Tasmania, Australia	Williams & Burreson 2006	DQ414301	DQ414255
			californiana	California, USA; from Scorpaena guttata = SG	Williams & Burreson 2006	DQ414304	DQ414258
			translucens	South Australia, Australia	Williams & Burreson 2006	DQ414306	DQ414260
		Bathybdella	*sawyer*	East Pacific Rise	Williams & Burreson 2006	DQ414311	DQ414265
		Malmiana	*buthi*	California, USA	Williams & Burreson 2006	DQ414322	DQ414276
		Myzobdella	*lugubris*	Virginia, USA = VA; freshwater	Williams & Burreson 2006	DQ414324	DQ414278
				Hawaii, USA = HI; freshwater	Williams & Burreson 2006	DQ414325	DQ414279
		Piscicolaria	*reducta*	Tennessee, USA	Williams & Burreson 2006	DQ414339	DQ414294
		Pterobdella	*amara*	Queensland, Australia	Williams & Burreson 2006	DQ414334	DQ414289

	Pontobdellinae*	*Zeylanicobdella*	*arugamensis de Silva*	Borneo	Williams & Burreson 2006	DQ414344	DQ414299
		Oxytonostoma	*typica*	Barents Sea, Finmarken Bank	Utevsky et al. 2007;Williams & Burreson 2006	EF405596	DQ414288
		Stibarobdella	*macrothela*	Heron Island, Australia = HER	Williams & Burreson 2006	DQ414340	DQ414295
				Hawaii, USA = HI	Williams & Burreson 2006	DQ414341	DQ414296
				Virginia, USA = VA	Williams & Burreson 2006	DQ414342	DQ414297
Arhynchobdellida Hirudiniformes	Hirudinidae						
		Limnatis	*nilotica*	Israel	Borda & Siddall 2004	AY425452	AY425470
		Macrobdella	*decora*	Michigan, USA	Siddall & Burreson 1998; Apakupakul *et al.* 1999	AF003271	AF116007

Appendix S4 Descriptive data for all COI and 18S sequences from non-turtle host Hirudinea taxa included in this study. Accession number is highlighted in yellow if the GenBank sequence was used for character-based DNA barcoding.

Appendix S5 Descriptive data for all Cheloniidae taxa and mtDNA control region sequences included in this study.

Family	Genus	Species	Locality	References	Genbank Accession Numbers
Cheloniidae					
	Caretta	*caretta*	Hutchinson Island, Florida	Encalada *et al*. 1998	AJ001074 & AJ001075
	Chelonia	*mydas*	Hutchinson Island, Florida	Lahanas *et al*. 1994 & Encalada *et al*. 1996; Allard *et al*. 1994	Z50124 & Z50125; M98394
			Atol das Rocas, Brazil	Encalada *et al*. 1996	Z50130-Z50133 & Z50135
			Sao Paulo, Ubatuba, Brazil	Naro-Maciel *et al*. 2007	DQ294212
			Hawaii	Dutton unpublished	AY540055-AY540057
			South China Sea, China	Duan & Gu unpublished	JF683409-JF683412
	Lepidochelys	*olivacea*	Baja California Sur, Mexico	Lopez-Castro & Rocha-Olivares 2005	AY920519-AY920523

Higher Taxon	Family (*subfamily)	Genus	Species	Locality	References	Genbank Accession Numbers
						28S
Rhynchobdellida						
	Glossiphoniidae					
		Glossiphonia	*complanata*	Mantle Lake, Maine, USA	Williams *et al*. 2013	JQ821578
		Helobdella	*stagnalis*	Västergötland, Sweden = SWD	Rousset *et al*. 2007	AY340402
				Mantle Lake, Maine, USA = USA	Williams *et al*. 2013	JQ821579
		Theromyzon	*tessulatum*	France	Borda & Siddall 2004	AY425404
	Piscicolidae					
	Piscicolinae*					
		Baicalobdella	*torquata*	Ukraine, Kharkiv Region, Pechenigy Reservoir	Utevsky *et al*. 2007	EF405573
		Branchellion	*torpedinis*	South Carolina, USA	Borda & Siddall 2004	AY425359
		Calliobdella	*lophii*	Norway	Utevsky *et al*. 2007	EF405584
		Caspiobdella	*fadejewi*	Ukraine, Kharkiv Region, Pechenigy Reservoir	Utevsky *et al*. 2007	EF405571
		Cystobranchus	*respirans*	Slovenia, Sava River near Ljubljana	Utevsky *et al*. 2007	EF405572
		Johanssonia	*arctica*	Newfoundland, Canada	Utevsky *et al*. 2007	EF405583
		Limnotrachelobdella	*okae*	Russia, Nevelskoy Strait	Utevsky *et al*. 2007	EF405585
		Nototheniobdella	*sawyeri*	Antarctica, Ross Sea, Terra Nova Bay	Utevsky *et al*. 2007	EF405588
		Piscicola	*geometra*	Ukraine, Siverskyi Donets River, Biol. Station of Kharkiv University	Utevsky *et al*. 2007	EF405574
		Trachelobdellina	*glabra*	Antarctica, Argentine Islands, Vernadsky Station, Galindez I., Marina Point	Utevsky *et al*. 2007	EF405587
	Platybdellinae*					
		Crangonobdella	*maculosa*	Russia, Tatar Strait	Utevsky *et al*. 2007	EF405580
		Heptacyclus	*virgatus*	Russia, Sakhalin, Aniva Bay	Utevsky *et al*. 2007	EF405579
		Notostomum	*laeve*	Barents Sea, Central Plateau	Utevsky *et al*. 2007	EF405581
			cyclostomum	Russia, Tatar Strait	Utevsky *et al*. 2007	EF405582
		Oceanobdella	*sakhalinica*	Russia, Sakhalin, Aniva Bay	Utevsky *et al*. 2007	EF405577
		Platybdella	*anarrhichae*	Platybdella anarrhichae Gusinaya Bank	Utevsky *et al*. 2007	EF405578
		Trulliobdella	*capitis*	Antarctica, Ross Sea, Terra Nova Bay	Utevsky *et al*. 2007	EF405576
	Pontobdellinae*					
		Megaliobdella	*cf. szidati*	Antarctica, Ross Sea	Utevsky *et al*. 2007	EF405569
			szidati	Ross Sea	Utevsky *et al*. 2007	EF405570
		Moorebdellina	*biannulata*	Ross Sea	Utevsky *et al*. 2007	EF405589
		Oxytonostoma	*typica*	Barents Sea, Finmarken Bank	Utevsky *et al*. 2007	EF405586
		Pontobdella	*muricata*	Slovenia, Gulf of Piran	Utevsky *et al*. 2007	EF405575
		Stibarobdella	*macrothela*	Virginia, USA	Borda & Siddall 2004	AY425403

Arhynchobdellida					
Hirudiniformes					
Hirudinidae					
	Hirudo	*orientalis*	Uzbekistan, Samarkand Province, Urgut District	Utevsky *et al*. 2007	EF405591
	Limnatis	*nilotica*	Israel	Borda & Siddall 2004	AY425389
	Macrobdella	*decora*	Michigan, USA	Borda & Siddall 2004	AY425390

Appendix S6 Descriptive data for all 28S sequences from non-turtle host Hirudinea taxa included in this study. Taxa highlighted in yellow if species served as an outgroup for character-based DNA barcoding.

	Position																	
	*		*	*	*			*							*			
Taxa	15	24	44	46	47	49	51	55	63	69	93	96	102	111	118	121	123	129
Ozobranchus margoi	**A**	T	**T**	**A**	**G**	**C**	**G**	**G**	**C**	**G**	**C**	**G**	T	**G**	**A**	A	T	**C**
Ozobranchus branchiatus CC/CM-FL	**A**	**C**	**T**	**A**	**G**	**T**	A	**G**	T	A	T	A	**C/** T	**G**	**A**	**G**	A	A
CM-BZ	**A**	**C**	**T**	**A**	**G**	**T**	A	**G**	T	A	T	A	T	A	**A**	**G**	**G**	A
CM-HI	**A**	**C**	**T**	**A**	**G**	**T**	A	**G**	T	A	T	A	T	T	**A**	**G**	**G**	A
CM-CHI	**A**	**C**	**T**	**A**	**G**	**T**	A	**G**	T	A	T	A	T	A	**A**	**G**	**G**	A
LO-MX	**A**	**C**	**T**	**A**	**G**	**T**	A	**G**	T	A	T	A	T	T	**A**	**G**	**G**	A

	Position														
						*			*						
Taxa	138	139	162	165	180	186	204	240	265	289	291	315	318	333	336
Ozobranchus margoi	**C**	T	A	**G**	T	**C**	T	T	**A**	G	A	**G**	A	**C**	**G**
Ozobranchus branchiatus CC/CM-FL	T	T/ **C**	**G/** A	A	**A**	**C**	T	**G**	**A**	**A**	T/ **C**	T	**C/** T	T	T
CM-BZ	T	**C**	A	A	**A**	**C**	T	**G**	**A**	**A**	T	T	T	T	T
CM-HI	T	**C**	**G/** A	A	**A**	**C**	**C**	**G**	**A**	**A**	T	T	T	T	T
CM-CHI	T	**C**	**G**	A	**A**	**C**	T	**G**	**A**	**A**	T	T	T	T	**C**
LO-MX	T	T/ **C**	A	A	**A**	**C**	T	**G**	**A**	**A**	T	T	T	T	T

	Position														
		*	*						*	*					
Taxa	342	353	360	366	369	393	444	480	481	482	484	517	519	534	537
Ozobranchus margoi	A	**G**	**T**	T	T	**C**	T	A	**C**	**G**	T	T	A	A	**T**
Ozobranchus branchiatus CC/CM-FL	**C/** T	**G**	**T**	**C**	A/ **G**	T	**C**	**G**	**C**	**G**	**C**	T/ **C**	A	**G/** A	A
CM-BZ	T	**G**	**T**	**C**	**G**	T	**C**	**G**	**C**	**G**	**C**	T	A	A	A
CM-HI	T	**G**	**T**	**C**	**G**	T	T	**G**	**C**	**G**	**C**	T	**G**	A	A
CM-CHI	T	**G**	**T**	**C**	**G**	T	**C**	**G**	**C**	**G**	**C**	T	**G**	A	A
LO-MX	T	**G**	**T**	**C**	**G**	T	T	**G**	**C**	**G**	**C**	T	**G**	A	A

	Position								
Taxa	546	549	570	579	595	624	627	642	648
Ozobranchus margoi	A	**G**	**C**	A	T	A	T	**G**	A
Ozobranchus branchiatus CC/CM-FL	A	A	T/ **C**	A	**C**	**G/** A	**C/** T	A	**G**
CM-BZ	**G**	A	T	**G**	**C**	A	**A**	A	**G**
CM-HI	A	A	T	**G**	**C**	A	**A**	A	**G**
CM-CHI	A	A	T	**G**	**C**	A	**A**	A	**G**
LO-MX	A	A	T	**G**	**C**	A	**A**	A	**G**

Appendix S7 Nucleotide positions selected if unique simple characteristic attributes (CAs) exist pertaining to the *Ozobranchidae* family. Pure CAs (bolded) are found solely in the *Ozobranchidae* family. Diagnostic sites with asterisks (*) above indicates the CAs were found in both *Ozobranchus* spp. species and in all specimens analyzed. Double solid lines separate the two morphologically distinct species of *Ozobranchus* spp., while the dash line represents the geographical barrier (Isthmus of Panama) separating the Atlantic and Pacific Ocean basin populations. At certain positions, pure CAs occur in only some members of a species, also known as private CAs. All private CAs unique to a particular species or certain members of the same species distinguished by turtle host or geographic location are color coded as follow: *O. margoi* (red), *O. branchiatus* (yellow), *O. branchiatus* CC-FL (blue), *O. branchiatus* CM-FL (green), *O. branchiatus* CM-BZ (pink), *O. branchiatus* CM-HI (violet), and *O. branchiatus* CM-HK (orange). No private CAs were identified for *O. branchiatus* collected from western Pacific *Lepidochelys olivacea* in Mexico. Simple CAs that help distinguish ocean basin origin are shaded grey.

CURRICULUM VITAE OF TRIET M TRUONG

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U.S. Citizen

EDUCATION

Wright State University, Dayton, Ohio **Advisor: Dr. Audrey E. McGowin**

- M.S., Chemistry (GPA 4.0) 2011-Present
  Thesis: "Investigating DNA barcoding potentials and genetic structure in *Ozobranchus* spp. from Atlantic and Pacific Ocean sea turtles"
- B.S., Chemistry (*magna cum laude*), Departmental Honors Scholar 2007-2011

Edison Community College, Piqua, Ohio

- English and Chemistry major, Dean's List 2005-2007

EXPERIENCE

Wright State University, Dayton, Ohio

Graduate Student Interviewer, Search Committee 2013

- Helped create, revised, and assembled interview questions
- Tabulated graduate students' ratings and submitted personal evaluation of each candidate applying for the Dean of the Graduate School

Grant Reviewer, Graduate Student Assembly 2013

- Served on Grant Committee that awarded $5000 in research and travel incentive grants to graduate students

Communications and Marketing, Chair, Graduate Student Assembly 2012-2013

- Manager and developer of the organization's website
- 1st Annual Yearbook creator and editor
- Served as GSA representative at the Graduate School Open House

Graduate Research Assistant, Chemistry Department 2013

- Co-PI on a chemical education study regarding the impact of computer simulations on learning in the entry-level chemistry laboratory
- Conducted PCR-based identification of leeches' gut content for viral analysis
- Analyzed the spatial heterogeneity of leeches from global sea turtle populations using mtDNA and rDNA markers

Graduate Teaching Assistant, Chemistry Department 2011- 2013

- Lab courses taught: Quantitative Analysis, Chemistry of Our World: Energy and the Environment, General Chemistry, Environmental Chemistry, and Selected Topics in Analytical Chemistry

Undergraduate Research Assistant, Chemistry Department 2009-2011

- Established a DNA barcode for identifying marine leeches using COI gene
- Analyzed and measured the 16 US EPA regulated polycyclic aromatic hydrocarbons in Austrian (*Pinus nigra*) needles using GC-MS

Chemistry Tutor, Student Academic Success Center 2008-2011

LexisNexis, Miamisburg, Ohio

Elsevier Data Analyst Intern 2011

- Developed rules to apply ontology to scientific articles section titles
- Utilized ScienceDirect in test environment for quality control purposes
- Tested ScienceDirect using mobile devices for informational inquiries

Kroger, Troy, Ohio and Springfield, Ohio

Nationally Certified Pharmacy Technician 2008-2012

Edison Community College Learning Center, Piqua, Ohio

English, Math, and Chemistry Tutor 2006-2007

Pro-Nails, Sidney, Ohio

Licensed Manicurist, Ohio State Board of Cosmetology 2005-2008

SKILLS

Editorial

- Technical writing: Standard operating procedures, research manuscripts and posters, technical reports and handouts/worksheets, laboratory quizzes and grading rubrics, magazine editor (Lifestyles Magazine, March 2007 issue)
- Languages: English and Vietnamese

Analytical Chemistry

- Instrumentation: IC, ICP-OES, GC-MS, LC-MS, HPLC
- Environmental methods: EPA Method 300.1, EPA Method 200.7, POCIS extraction and analysis, water quality sampling, microbiological water analysis

Molecular Biology

- Equipment: PCR thermocycler, ND-1000 NanoDrop Spectrophotometer, centrifuge, Class II Biological Safety Cabinet, microscope camera
- Techniques: DNA extraction and purification, PCR, gel electrophoresis, sequencing
- Computer software: MEGA5, DnaSP5, Network, zt, Alleles in Space, Sequencher

HONORS

3rd place-graduate poster presentation, Cleveland State Research Conference	2013
WSU Chemistry Department Outstanding Graduate Teaching Award	2013
WSU Graduate Student Assembly Members' Choice, Exceptional Performance Award	2013
Outstanding graduate poster presentation, Annual Dayton Local ACS Poster Event	2013
WSU Graduate Student Assembly Member of the Month	2013
Featured in Winter issue article of Environmental Monitor	2013
Graduate Student Award, ACS Division of Environmental Chemistry	2013
2nd place-graduate oral presentation, Cleveland State Research Conference	2012
Best undergraduate research poster, WSU Chemistry Department Posters in the Hall	2011
3rd place-undergraduate poster award, Carnegie Mellon University	2010
Edison Community College Top Ten Writing Award	2006

CERTIFICATIONS

Wright State University, Dayton, Ohio

Department of Environmental Health and Safety

- Biological safety training 2010 & 2012
- Radiation safety awareness training 2012
- Hazardous communication training 2012
- Hazardous waste training 2012
- Lab safety training 2012

Pharmacy Technician Certification Board 2008-Present

Edison Community College Learning Center, Piqua, Ohio

College Reading and Learning Association

- Completed Regular/Level I Tutor training 2007

VOLUNTEER EXPERIENCE

International Sea Turtle Society 33rd Annual Symposium on Sea Turtle Biology and Conservation, Baltimore, Maryland 2013

- Door attendant at the Terrapin, Tortoise & Freshwater session
- Provided customer service at the registration table
- Sold ISTS fundraising items (e.g., t-shirts, memorabilia, etc.)
- Desk attendant and sign-up assistant for the Speed Chatting Workshop
- Part of the symposium clean-up crew after the event ended

Wright State University Chemistry Club 2010-2011

- Participated at the May Daze festival to raise money for breast cancer and to help recruit new members to the organization

PROFESSIONAL MEMBERSHIP

American Chemical Society, Environmental Chemistry Division 2010-Present

GRANTS/SCHOLARSHIPS

Awarded

- Wright State University Graduate Student Assembly Professional Development Grant, 33rd Annual Symposium on Sea Turtle Biology and Conservation, $400. February 2-8, 2013, Baltimore, MD.
- 2013 Travel Grant, International Sea Turtle Society, 33rd Annual Symposium on Sea Turtle Biology and Conservation, $1169.09. February 2-8, 2013, Baltimore, MD.
- 2012 Travel Award, Graduate Research in Biological Chemistry, 244th American Chemical Society National Meeting & Exposition, $500. August 19-21, 2012, Philadelphia, PA.
- Wright State University Graduate Student Assembly Original Work Grant. Triet M. Truong, PI; *Investigation of Sea Turtle Leeches as a Mechanical Vector in the Transmission of Sea Turtle Fibropapillomatosis*. $750. 1/2012-12/2013
- Choose Ohio First Scholarship. $1500 per fiscal year (full-time enrollment). 2011-Present.
- Edison Community College Minority Scholarship. Full-tuition. 2006-2010.

Submitted

- Great Lakes National Scholarship Program. Graduate Students in Science, Technology, Engineering, and Mathematics. $2,500. May 11, 2011 & April 22, 2013.
- Wright State University Graduate Student Assembly Professional Development Grant, 244th American Chemical Society National Meeting & Exposition in Philadelphia, PA. $400. March 26, 2012.
- Wright State University Friends of the Libraries' Undergraduate Library Research Award. Triet M. Truong, undergraduate researcher; *Identifying new haplotypes for sea turtle leeches using DNA barcoding*. $1000. March 9, 2011.
- Wright State University Graduate Council Scholarship. Master of Science in Chemistry. Full tuition and stipend. February 10, 2011.
- Wright State University Summer Undergraduate Research. Triet M. Truong, undergraduate researcher; *Investigation of sea turtle leeches as vector organisms in the transmission of Fibropapilloma-Associated Turtle Herpesvirus*. $4500 for student. $500 for faculty. March 13, 2010.

PUBLICATIONS

- Kammler DC, **Truong TM**, VanNess G, McGowin AE. A Service-Learning Project in Chemistry: Environmental Monitoring of a Nature Preserve. *Journal of Chemical Education* (2012), 89, 1384–1389. ***(Project on the cover of journal)***

- Lavretsky P, **Truong TM**, McGowin AE, Balazs GH, Peters J. New primers reveal the presence of a duplicate histone H3 in the marine turtle leech *Ozobranchus branchiatus*. *Conservation Genetics Resources* (2012), 4, 487-490.

- Tomashuk TA, **Truong TM**, Madhavi M, McGowin AM. Atmospheric polycyclic aromatic hydrocarbon profiles in pine needles and particulate matter and their temporal variations in Dayton, Ohio, USA. *Atmospheric Environment* (2012), 51, 196-202.

- McGowin AE, **Truong TM**, Corbett AM, Bagley DA, Ehrhart LM, Bresette MJ, Weege ST, Clark D. Genetic barcoding of marine leeches (*Ozobranchus* spp.) from Florida sea turtles and their divergence in host specificity. *Molecular Ecology Resources* (2011), 11, 271–278. ***(Research on the cover of journal)***

PRESENTATIONS

Lecture Presentation

- 7th Annual Cleveland State Interdisciplinary Research Conference. “Investigating species complexity and host-parasite co-evolution in *Ozobranchus* spp. from Atlantic and Pacific Ocean sea turtles.” **Triet M Truong**, Audrey E McGowin, Jeffrey L Peters, Philip Lavretsky. Cleveland State University. Cleveland, OH. October 12, 2013

- Wright State University 2013 Eco-Seminar. “DNA barcoding: A novel tool for investigating species complexity and host-parasite co-evolution in *Ozobranchus* spp. from Atlantic and Pacific Ocean sea turtles.” **Triet M Truong**, Audrey E McGowin, Jeffrey L Peters, Philip Lavretsky. Wright State University. Dayton, OH. September 4, 2013

- Midwest Ecology and Evolution Conference. "Establishing a global DNA barcode for *Ozobranchus* spp. in the Atlantic and Pacific Oceans." **Triet M Truong**, Audrey E McGowin. University of Notre Dame. South Bend, IN. March 24, 2013

- 6th Annual Cleveland State Interdisciplinary Research Conference. "Establishing a global character-based DNA barcode for marine leeches (*Ozobranchus* spp.) from sea turtles in the Atlantic and Pacific Ocean basins." **Triet M Truong**, Audrey E McGowin. Cleveland State University. Cleveland, OH. November 3, 2012 ***Awarded 2nd place-graduate oral presentation***

- Wright State University Chemistry Department Seminar. "Custom oligonucleotide synthesis: an organic process for assembling the building blocks of life." **Triet M Truong**, Audrey E McGowin. Wright State University. Dayton, OH. September 14, 2012

- Choose Ohio First STEMM Scholar Showcase. "A global collaboration to save the world's sea turtles right here in southwestern Ohio." **Triet M Truong**, Audrey E McGowin. Center of Science and Industry. Columbus, OH. January 23, 2011

- Wright State University 2011 Eco-Seminar. "Solving the fibropapillomatosis pandemic through various interdisciplinary lens." **Triet M Truong**, Audrey E McGowin, Jeffrey L Peters, Philip Lavretsky. Wright State University. Dayton, OH. October 13, 2011

- Wright State University 2010 Eco-Seminar. "Identification of sea turtle leeches using DNA barcoding." Audrey E McGowin, **Triet M Truong**, Adrian M Corbett. Wright State University. Dayton, OH. October 6, 2010

- Wright State University Honors Research Colloquium PowerPoint Presentation. "Identification of sea turtle leeches using DNA barcoding." **Triet M Truong**, Audrey E McGowin, Adrian M Corbett. Wright State University. Dayton, OH. May 27, 2010

Poster Presentation

- 7th Annual Cleveland State Interdisciplinary Research Conference. "Monitoring the Health of Glen Helen Nature Preserve: Fall 2012 Sediment and Water." **Triet M Truong**, Audrey E McGowin. Cleveland State University. Cleveland, OH. October 12, 2013 ***Awarded 3rd Place at the poster competition***

- 246th American Chemical Society National Meeting & Exposition. "Genetic identification of *Ozobranchus* spp. from Atlantic and Pacific Ocean sea turtles for host parasite studies." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Indiana Convention Center. Indianapolis, IN. September 10, 2013

- Choose Ohio First STEMM Scholar Showcase. "Establishing a global DNA barcode for marine leeches (*Ozobranchus* spp.) from sea turtles in the Atlantic and Pacific Oceans." **Triet M Truong**, Audrey E McGowin. Columbus State Community College. Columbus, OH. March 15, 2013

- Choose Ohio First STEMM Scholar Showcase. “Monitoring the Health of Glen Helen Nature Preserve: Fall 2012 Sediment and Water.” **Triet M Truong**, Audrey E McGowin. Columbus State Community College. Columbus, OH. March 15, 2013

- 4th Annual Wright State University Celebration of Research, Scholarship and Creative Activities. “Establishing a global DNA barcode for marine leeches (*Ozobranchus* spp.) from sea turtles in the Atlantic and Pacific Oceans.” **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Wright State University. Dayton, OH. April 12, 2013

- 4th Annual Wright State University Celebration of Research, Scholarship and Creative Activities. “Monitoring the Health of Glen Helen Nature Preserve: Fall 2012 Sediment and Water.” **Triet M Truong**, Ted Alfred, Laura Bailey, Kelsi Eberst, Tara Fujimoto, Aaron Lampert, Kyle Liddy, Sarah Sharrock, Joseph Solch, Garret VanNess, and Audrey E. McGowin. Wright State University. Dayton, OH. April 12, 2013

- Annual American Chemical Society/Society for Applied Spectroscopy Poster Session. “Establishing a global DNA barcode for marine leeches (*Ozobranchus* spp.) from sea turtles in the Atlantic and Pacific Oceans.” **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Wright State University. Dayton, OH. March 12, 2013

- Annual American Chemical Society/Society for Applied Spectroscopy Poster Session. “Monitoring the Health of Glen Helen Nature Preserve: Fall 2012 Sediment and Water.” **Triet M Truong**, Ted Alfred, Laura Bailey, Kelsi Eberst, Tara Fujimoto, Aaron Lampert, Kyle Liddy, Sarah Sharrock, Joseph Solch, Garret VanNess, and Audrey E. McGowin. Wright State University. Dayton, OH. March 12, 2013

- 33rd Annual Symposium on Sea Turtle Biology and Conservation. “Establishing a global DNA barcode and molecular phylogeny for marine leeches (*Ozobranchus* spp.) from sea turtles in the Atlantic and Pacific Ocean basins” **Triet M Truong**, Philip Lavretsky, Jeffrey L Peters, and Audrey E McGowin. Baltimore Marriot Waterfront Hotel. Baltimore, MD. February 2-8, 2013

- 33rd Annual Symposium on Sea Turtle Biology and Conservation. “Heavy metal analysis of marine leeches (*Ozobranchus* spp.) to assess exposure in sea turtles” Kyle Danielson, Jacqueline Roth, **Triet M Truong**, Audrey E McGowin. Baltimore Marriot Waterfront Hotel. Baltimore, MD. February 2-8, 2013

- 6th Annual Cleveland State Interdisciplinary Research Conference. “Establishing a global DNA barcode and molecular phylogeny for marine leeches (*Ozobranchus* spp.) from sea turtles in the Atlantic and Pacific Ocean basins ” **Triet M Truong**, Philip Lavretsky, Jeffrey L Peters, and Audrey E McGowin. Cleveland State University. Cleveland, OH. November 3, 2012

- Ohio Valley Regional Chapter of the Society of Environmental Toxicology and Chemistry. “Trace metal analysis in sea turtle leeches (*Ozobranchus* spp.) using ICP-

OES." Kyle Danielson, Jacqueline Roth, **Triet M Truong**, Audrey E McGowin. Ohio Miami University. Oxford, OH. September 28, 2012

- Summer Science Research Symposium. "Heavy metal analysis in sea turtle leeches (*Ozobranchus* spp.) using ICP-OES." Jacqueline Roth, Kyle Danielson, **Triet M Truong**, Audrey E McGowin. Ohio Wesleyan University. Delaware, OH. September 17, 2012

- 244th American Chemical Society National Meeting & Exposition. "Establishing a global DNA barcode and molecular phylogeny for identifying marine leeches (*Ozobranchus* spp.) from sea turtles in the Atlantic and Pacific Ocean basins." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Pennsylvania Convention Center. Philadelphia, PA. August 21, 2012

- Wright State University Chemistry Department Posters in the Hall. "New Primers Reveal the Presence of a Duplicate Histone H3 in the Marine Turtle Leech *Ozobranchus branchiatus*." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Wright State University. Dayton, OH. June 1, 2012

- 3rd Annual Wright State University Celebration of Research, Scholarship and Creative Activities. "Identifying new haplotypes and potential cryptic species for marine leeches (*Ozobranchus* spp.) from Hawaiian and Florida sea turtles based on molecular data." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Wright State University. Dayton, OH. April 13, 2012

- 3rd Annual Wright State University Celebration of Research, Scholarship and Creative Activities. "Monitoring the health of Glen Helen Nature Preserve: Can I drink the water?" **Triet M Truong**, Richard Cooke, Jessica Dagher, Perri Freeman, Felicia Gooden, Richard Grimes, Nargees Jumathan, Jeremy Lear, Kayla Lilly, Ryann Patrus, Zeb Reichert, Morgan Russell, Sam Senzek, Garret VanNess, David Kammler, and Audrey E McGowin. Wright State University. Dayton, OH. April 13, 2012

- Annual American Chemical Society/Society for Applied Spectroscopy Poster Session. "Identifying new haplotypes and potential cryptic species for marine leeches (*Ozobranchus* spp.) from Hawaiian and Florida sea turtles based on molecular data." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Central State University. Wilberforce, OH. April 3, 2012

- Annual American Chemical Society/Society for Applied Spectroscopy Poster Session. "Monitoring the health of Glen Helen Nature Preserve: Can I drink the water?" **Triet M Truong**, Richard Cooke, Jessica Dagher, Perri Freeman, Felicia Gooden, Richard Grimes, Nargees Jumathan, Jeremy Lear, Kayla Lilly, Ryann Patrus, Zeb Reichert, Morgan Russell, Sam Senzek, Garret VanNess, David Kammler, and Audrey E McGowin. Central State University. Wilberforce, OH. April 3, 2012

- Midwest Ecology and Evolution Conference. "Identifying new haplotypes and potential cryptic species for marine leeches (*Ozobranchus* spp.) from Hawaiian and Florida sea turtles based on molecular data." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. University of Cincinnati. Cincinnati, OH. March 24, 2012

- 42st Meeting of the American Chemical Society Central Region. "Identifying new haplotypes and potential cryptic species for marine leeches (*Ozobranchus* spp.) from Hawaiian and Florida sea turtles based on molecular data." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. University Place Conference Center. Indianapolis, IN. June 10, 2011

- Wright State University Chemistry Department Posters in the Hall. "Identifying new haplotypes and potential cryptic species for marine leeches (*Ozobranchus* spp.) from Hawaiian and Florida sea turtles based on molecular data." **Triet M Truong**, Audrey E McGowin, Philip Lavretsky, Jeffrey L Peters. Wright State University. Dayton, OH. June 3, 2011 ***Awarded best undergraduate research poster***

- Wright State University Chemistry Department Posters in the Hall. "DNA barcoding of sea turtle leeches (*Ozobranchus* spp.) in Florida coastal waters." **Triet M Truong**, Audrey E McGowin. Wright State University. Dayton, OH. June 3, 2011

- 2nd Annual Wright State University Celebration of Research, Scholarship and Creative Activities. "DNA barcoding of sea turtle leeches (*Ozobranchus* spp.) in Florida coastal waters." **Triet M Truong**, Audrey E McGowin. Wright State University. Dayton, OH. April 8, 2011

- 75th Annual Meeting of the Florida Academy of Sciences. "DNA barcoding of sea turtle leeches (*Ozobranchus* spp.) in Florida coastal waters." Audrey E McGowin, **Triet M Truong**. Florida Institute of Technology. Melbourne, FL. March 11, 2011

- Annual American Chemical Society/Society for Applied Spectroscopy Poster Session. "DNA barcoding of sea turtle leeches (*Ozobranchus* spp.) in Florida coastal waters." **Triet M Truong**, Audrey E McGowin. University of Dayton. Dayton, OH. March 1, 2011

- Carnegie Mellon University Bridging Research Communities. "Identification of sea turtle leeches using DNA barcoding." **Triet M Truong**, Audrey E McGowin, Adrian M Corbett. Carnegie Mellon University. Pittsburgh, PA. October 16, 2010 ***Awarded 3rd Place at the poster competition***

- 41st Central Regional Meeting of the American Chemical Society. "Identification of sea turtle leeches using DNA barcoding." **Triet M Truong**, Audrey E McGowin, Adrian M Corbett. Dayton Convention Center. Dayton, OH. June 17, 2010

- 1st Annual Wright State University Celebration of Research, Scholarship and Creative Activities. "Identification of sea turtle leeches using DNA barcoding." **Triet M Truong**, Audrey E McGowin, Adrian M Corbett. Wright State University. Dayton, OH. April 16, 2010

- Wright State University Chemistry Department Posters in the Hall. “Identification of sea turtle leeches using DNA barcoding.” **Triet M Truong**, Audrey E McGowin, Adrian M Corbett. Wright State University. Dayton, OH. June 4, 2010

- Wright State University Chemistry Department Posters in the Hall. “Analysis of Atmospheric PAHs in the Austrian Pine.” Timothy Tomashuk, **Triet M Truong**, Audrey E McGowin. Wright State University. Dayton, OH. June 5, 2009